A STUDY OF ENDOMYCES CAPSULATUS REWBRIDGE, DODGE AND AYERS: A CAUSATIVE AGENT OF FATAL CEREBROSPINAL MENINGITIS[1]

MORRIS MOORE

Formerly Rufus J. Lackland Research Fellow in the Henry Shaw School of Botany of Washington University

Introduction

The probable taxonomic relationships of the fungi known as yeast-like organisms have for many years attracted much attention and discussion among mycologists, and to a certain extent among medical men. Considerations of their general physiological properties, modes of reproduction, their cytological differentiations, and varied pathogenic abilities were involved. The group comprises several families and many genera and species, with subdivisions of these, which from time to time call forth long and expounding dissertations on the mechanism of reproduction (the perfect stage). The problem of classification is unfortunately a very complex one, since it has been found, at least in the present work and with other closely related fungi which will be considered later, that several forms of development may be present in the same culture. Such phenomena are rather rare but render incorrect any means of classification based on one phase only.

It is well known that the fungi are divided, on the basis of their morphology, cytology, and sexual development, into three major divisions: Phycomycetes, Ascomycetes, and Basidiomycetes, and to these is appended a fourth group, the Fungi Imperfecti, the life history of which is not at all or incompletely known. The yeast-like organisms are considered to constitute a branch of both the Ascomycetes and the Fungi Imperfecti, the former where the production of asci is found, and the latter where no sexual development has been determined. More specifically, the perfect forms are considered to be members of the Endomycetaceae (Gaümann and Dodge, '28; Rewbridge,

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Dodge, and Ayers, '29; Moore, '33), and Coccidioideaceae (Moore, '32), including such genera as *Endomyces*, *Coccidioides*, *Rhinosporidium*, and *Pseudococcidioides*, while the imperfect forms, as *Monilia*, *Cryptococcus*, *Oidium*, *Mycoderma*, and several others have features which are somewhat similar to the above groups. This of course does not include the true yeasts which are included under the Saccharomycetaceae.

It was because of the imperfect knowledge existing in the field that the author undertook the study of the cytology of *Endomyces capsulatus*, which had been isolated from a case of meningo-encephalitis. During the course of the work it was found desirable to learn more about the phenomena which might be associated with the pathogen, and hence attention was paid to its physiological and other properties. In addition, the occurrence of other infections by closely allied organisms, *E. capsulatus* var. *isabellinus* (Moore, '33), and *E. dermatitidis* (Moore, '33a), have yielded the fungi which have been used in a comparative study, so that what may pertain to *E. capsulatus* has been found to hold true for the latter, with some minor exceptions as chromogenesis and cultural characteristics.

HISTORICAL REVIEW

Occasionally debatable points in the field of medical mycology are cleared up by the isolation of some fungus. Such an organism, *E. capsulatus*, was cultured in 1928 from a case of meningitis. The patient was a white, married male, 48 years of age, a furrier by trade. He entered the Boston City Hospital in August, 1928, complaining of having had continuous headaches in the frontal and temporal lobes for a period of three and a half months. His illness began with a hard, tender, slightly movable mass on the medial surface of the lower third of the left thigh, about five months before entry. This mass subsided within four weeks, but a similar mass then appeared beneath the anterior surface of the neck. He developed defects in memory, losing himself on familiar streets, and could not keep track of time. He lost interest and was unable to understand questions or conversation.

The cyst which had been present at the level of the thyroid isthmus between the trachea and anterior border of the sterno-

cleidomastoid muscle was aspirated. Thirty cc. creamy, tenacious, yellow pus was obtained, from which *E. capsulatus* was cultured. Similar organisms were obtained from the pus aspirated from the nodule of the left thigh.

About a month later, the patient's condition became worse. His temperature increased rapidly, as did his pulse and respiration, and he finally died. The clinical diagnosis was mycotic meningo-encephalitis (?), and an X-ray diagnosis showed bilateral pulmonary tuberculosis. It has been the author's experience that an infection of this sort which involves the lungs is often mistaken for tuberculosis, and the diagnosis made here may have been incorrect. The organism from this case has been used in these studies for the greater part, having been kept as a stock culture by alternate transfers on nutrient and Sabouraud's agar.

The second case due to the variety *isabellinus* (pl. 21) occurred in St. Louis and was reported by MacBryde and Thompson. The patient, a white male, 28 years of age, had been a plumber at the time of the appearance of the skin lesions. He was first admitted to the Barnard Free Skin and Cancer Hospital in February, 1930, with a palm-sized annular ulceration on the left forearm just above the wrist, which involved the dorsal surface chiefly and showed clinical signs of blastomycetic dermatitis. Endomycetes were isolated from the pus of this lesion. About 3 cm. to the right of the anus was another lesion about 3 cm. in diameter and involving the anal margin. On the upper part of the right arm was a scar of a lesion which had healed spontaneously. All active lesions were pruriginous and had a foul smell. The patient later, in January, complained of coryza, with a mild cough, pain in the right temporal region, accompanied by a rise in temperature. His headache became universal and intense, posterior to the eyes and extending to the occiput and base of the neck. There was a marked bilateral Kernig reflex action. He had nauseation and vomiting spells. A lumbar puncture gave a cloudy fluid under slight pressure, in which were seen the organisms found in greater abundance with later punctures. At this time he received a large dose of iodine solution which cleared the spinal fluid and decreased the number of budding cells, improving

his health greatly. However, two weeks later he had a relapse, became comatose, and three days before death developed three small subcutaneous abscesses. One was on the right wrist, one on the sacrum, and one on the left side of the chest, and all contained pus and *E. capsulatus* on culture. The patient died forty-six days after the onset of the meningitis symptoms and about three years after the first appearance of the skin lesions.

The morphology and cytology of the organism of this case was found to be the same as the first fungus, having a very light cinnamon color as contrasted with the hyaline or white, in a mass, of *E. capsulatus*, and cultural characteristics similar in detail.

The third organism studied is the one which has gone under many generic names, as *Blastomyces*, *Oidium*, *Saccharomyces*, *Cryptococcus*, and *Mycoderma*, because its life history was incompletely known and the sexual act was not definitely established, or the presence of the ascus and the actual number of spores determined. Historically this fungus is important because it causes a disease known as blastomycosis which has become fairly widespread, both in this country and Europe. The organism studied by the author was isolated from a case which occurred at the Barnard Free Skin and Cancer Hospital of St. Louis and has been dealt with in detail in a previous paper (Moore, '33). Suffice it to say here that *E. dermatitidis*, the organism causing a clinical condition known as blastomycosis, is similar to the above organisms, differing in its chromogenesis, being a dark cinnamon to brown in culture and having a somewhat different cultural reaction, to be mentioned later.

In addition to the above three forms, an organism obtained from a case of the so-called European blastomycosis or generalized torulosis reported by Urbach and Zach ('30) (pl. 19, figs. 13–25) has been studied to a certain extent. The case in brief was that of a 27-year-old shoe-worker who had never left Europe. He entered the University Clinic for Syphilology and Dermatology at Vienna with a swelling of the gums of the lower jaw which was excised, and a half year later he returned with a similar swelling of the gums of the upper jaw. About one year later the patient noticed an abscess-like swelling on the left side of the abdominal region, two months later a similar disorder on

his left thigh, and eight weeks later the same thing was found on his neck. The lesion on his left thigh was aspirated and from it 200 cc. purulent substance was obtained. A short time after that an inflammation of the lungs developed which healed spontaneously, but left him with a cough and vomiting spells. Yeast-like cells were cultured from the purulent secretion of the left thigh.

The disease was clinically identical with syphilis, tuberculosis, leukemia, and several others. Inoculation of an extract of the yeast-like cells (blastomycin) brought forth strong local, focal, and generalized reactions, giving evidence of the presence of blastomycosis.

The lesions were somewhat superficially healed by treatment. The patient later developed high temperatures in the evening, oral pains, deafness, violent coughing at night, and ruby-red expectorations. A tumor of the nasal septum, very suggestive of rhinosporidiosis, had developed and a catarrh of the Eustachian tube as a result of the tumor. There was an ulceration of the right tonsil and other lesions close by, and finally a heavy exudate in his lungs. Treatment gave him relief temporarily, but he returned to the clinic a short time later with a continuous headache, high temperature, and rapid spread of the mouth lesions. Paralysis of the optic musculature set in, with unconsciousness, and death ensued.

The organism from this case has been cultured and subcultured and as yet has shown no final stage as is present in the three above forms. However, some organisms require a long time before the perfect stage is obtained. Furthermore, the histological and pathological condition of the patient as investigated by Chiari ('30), shows the identical tissue reaction present in cases of *E. dermatitidis*.

With this brief review of the history of the organisms investigated, let us now turn to a study of *E. capsulatus*.

Technique

E. capsulatus was kept growing by subculturing on nutrient and Sabouraud's agar as a stock culture. In the work done here, the organism was grown on nutrient agar, a product of the

Digestive Ferments Co., at pH 6.8. In cases where an abundant growth was necessary, with a thick mycelium, Sabouraud's agar, pH 5.6, was employed.

For studying the cytology with regard to the nuclear changes in the sexual act, glycerine agar (beef extract agar plus 6 per cent glycerine, pH 7.1) was used. This medium gave the greatest number of asci in culture, in addition to a thick growth and many important diagnostic features, as chlamydospores, conidia, racquet mycelium, etc. Cultures on nutrient agar and Sabouraud's agar were also used, but the cells were not so good for details as on the above medium.

The cultures were fixed with a number of agents. Flemming's stronger solution shrunk the material rather noticeably, but the weaker did not give very good results either. Bouin's picro-formalin solution, which has been advocated by Kater ('27) and other cytologists working with yeasts, was of no value in this work. Benda's fluid, which is a modification of Flemming's stronger solution, caused the fungus to have too great an affinity for the stains, and inasmuch as it is difficult to destain the organism without causing some sort of damage, it had to be used with caution. Hermann's fluid, which is probably the most expensive of the fixing agents, was also used. This is a variation of Flemming's fluid, the chromic acid being replaced by platinic chloride, and although not particularly recommended by Chamberlain ('32), the author has found it to give the best results. Various other fixing agents were employed, but the results were not worthy of note.

The embedding proved to be a problem because the material had to be fixed and embedded while on the agar. Paraffin was used at first, but in the glycerine agar cultures it would not adhere to the agar substrate and sectioning could not be done without damage to the material. Gradual and repeated changes of paraffin did not remedy the condition. A medium was then tried which contained very little protein and carbohydrate, but no suitable growth could be obtained. Finally the cultures were embedded in Dupont parlodion, using the Jeffrey technique. This method has been recently outlined by Wetmore ('32) and with some modification was applied here. It consists in fixing

the material, in this case either with Hermann's or Benda's fluid, pumping and dehydrating, then passing it through an intermediate stage of ether-alcohol, and then through a series of concentrations of the celloidin or parlodion. The first concentration was a 2 per cent solution and each series increased 2 per cent until the final concentration was 12 per cent. The material on the agar was taken from the test-tube after fixing, cut into convenient pieces, and put through the above procedure. The pieces were kept in a tightly plugged bottle in an incubator at 45° C. and changes were made daily. After embedding, sections were cut to a thickness of 10 μ. These were then stained and mounted.

The best stain for this procedure was found to be iron-alum haematoxylin, using Heidenhain's haematoxylin, although Ehrlich's gave just as good results. A combination of Benda's fixing agent and iron-alum haematoxylin showed the reticulated network and the metachromatic material very well, whereas Hermann's fluid plus Heidenhain's iron-alum haematoxylin was best for nuclear structure. Methylene blue and Hermann's fluid brought out the vacuoles and volutin and the metachromatic material very clearly, as seen in pl. 23, figs. 15–18.

For morphological work, hanging-drop cultures were made of 2 per cent proteose peptone and 2 per cent bacto-peptone, as well as lactose broth cultures. Material was also placed in a drop of a 1 per cent solution of crystal violet (aqueous) in glycerine, the dye being added to the desired intensity. The preparation was allowed to stand from 15 to 30 minutes for a sufficient clearing. Aman's lactophenol was also applied, as was carbol fuchsin and very dilute solutions of methylene blue, eosine, and crystal violet. In addition, iodine potassium iodide (saturated solution) was used for studying the glycogen contents of the cells and chondriosomes, as advocated by Guilliermond. Osmic acid, platinic chloride, and iodine green were also used, as well as neutral red, but these materials and other methods of procedure will be explained later in the text.

Cytology

The cytology of the lower Ascomycetes, particularly the yeasts

and the yeast-like organisms, had been for a number of years a topic of discussion among the older cytologists. The question as to whether or not a nucleus was present was indeed a serious one, judging by the numerous and lengthy dissertations on the subject. As is customary, there were two sides to the argument. One group maintained that the cells, of the yeasts in this case, were made up of a mass of protoplasm and of nuclein, without a true nucleus, and that the nuclein is differentiated at times in the cytoplasm in the form of granules which assume a definite color on staining. The other group favored the presence of a definite nucleus.

The first reference to a nucleus in yeasts was perhaps made in 1844 by Nägeli, who decided that "a little nucleus of whitish mucus, lying on the membrane, regularly in each cell" (Wager, '98), was often found in the yeast cell. Whether Nägeli actually saw a nucleus in the fresh condition is rather doubtful, for an oil globule has often been mistaken for the nucleus even to-day, with our more advanced knowledge of cytology.

About five years later, in 1849, Schleiden, by treating cells with ether-alcohol or potash, was able to find a rounded structure with a clear cell wall which contained delicate granules either singly or in groups, and in addition, a large flat body, which he called a cytoblast.

Following this work, Brücke, in 1861, asserted that in living material, as well as dead cells treated with iodine and acetic acid, no definite nucleus could be seen and he reprimanded the former workers by saying that no one was justified in taking bodies of various sizes and numbers, such as often occur, for nuclei.

In 1879 Schmitz, using haematoxylin, was able to demonstrate a nucleus in the cytoplasm of the cell near the vacuole, while Strasburger, repeating this work in 1884 and 1889, using picric acid and haematoxylin, was able to confirm these observations, finding that the nucleus was not demonstrable in unstained material.

Krasser, in 1885, contradicted these findings by saying that granules existed in the yeast-cell, but no nucleus. His main argument was that there was no specific staining reaction for nuclei, and furthermore that the absence of a definite nucleus

in the yeast-cell was supported by the rapid growth of the organism. For this reason he believed that there was nuclein in the cell which was distributed through the protoplasm very much as is generally held to be the case with bacteria to-day.

Other workers who held the view that a nucleus, or at least a "corpuscle" as some called it, was present, the biochemical properties and physiological functions of which were analogous to those found in the cells of plants and of animals, were Hansen, Strasburger, Zacharias, Moeller, Buscalioni, Henneguy, Dangeard ('93), Janssens ('02), and Janssens and Leblanc ('98). In fact, the latter writers made the following statement, "*La cellule de la levure peut être considérée comme formé sur la type général de la cellule.* On y trouve en effet, un *noyau*, un *protoplasme* et *une membrane.*" To these workers the nucleus had a vacuolar appearance with a very differentiated structure, to others it was a homogeneous body. Wager ('98), in a detailed bit of work, described it as being a vacuole filled with chromatin granulations (perhaps somewhat as is shown in pl. 23, figs. 17–18) which had been taken for a nucleus by Janssens and Leblanc, and furthermore that a spherical and homogeneous body, considered by some as the nucleus, was always adjacent to the vacuole and could be compared with a nucleolus. He considered the vacuole plus the eccentric nucleolus as a primitive stage in the phylogeny of the development of the nucleus. This belief was also based on the observation that the small body and the vacuole divide simultaneously by budding.

Guilliermond, in 1902, demonstrated that a definite nucleus was present, and that the vacuole was independent of the nucleus, being filled with granulation products which at the present time he holds to be nuclear decomposition substances (nucleic acid derivatives with some unknown base). For the granules he retained the name "corpuscules métachromatiques," a term which had been applied previously and which is used even now.

Those who denied the presence of a nucleus but admitted the presence of a nuclein substance as described above were Raum, Roncali, Hieronymus, Macallum, and others.

In accordance with the views of the older writers, the author has found that in *Endomyces* as treated here, when first isolated

from the tissue of the host, no nucleus exists, but simply a distribution of the chromatin material or nuclein or metachromatic corpuscles throughout the cell, as may be seen in pl. 19, figs. 13–14, pl. 20B, pl. 21, figs. 1–3, and several others. This feature is a quite constant character of freshly isolated cells. However, when kept on agar or an artificial substrate, the cells not only change their form, passing through what may be termed the secondary stage, that is changing from a yeast-cell to a hyphal form, but also undergo a change in nucleoplasmic make-up. It has been found that even though the yeast-like cells retain the same morphological characteristics on agar media, their chromatin material or nuclein is converted into a definite nucleus. This was clearly shown to be the case in *E. dermatitidis* (Moore, '33). What the mechanism involved here might be, it is difficult to say, but we may conjecture that it is linked up with the adaptation to a changed environment and a different mode of development to which it must become accustomed, as shall be pointed out later.

With such views being held in the past, it was of course to be expected that the presence of any phenomena which might involve the nucleus, as mitosis, would also be in dispute. In yeasts the question of nuclear division has received considerable attention, particularly by Guilliermond, Dangeard, and several others, the former writing many and long papers on the subject, and it has been said by some present-day workers, and the following is a direct quotation from the papers of one of them, that "the ideas of Guilliermond . . . which gain weight by the mere bulk of his work on yeast, seem to meet with more favor."

The problem of the division of the nucleus in yeasts is perhaps more complex than would seem offhand. The same writers who held that a nucleus was present in the cell first described nuclear phenomena. Wager ('98) interpreted an amitosis in the yeast with perhaps evidence of chromosomes. Janssens and Leblanc ('98), working with *Saccharomyces cerevisiae* and *S. Ludwigii*, as well as *Schizosaccharomyces octosporus* and *S. Pombe*, described a form of mitosis which, in the light of other works, seemed very suspicious and they made the following statement, "On peut dire que tout ce qui diminue la vitalité d'une cellule tend à réduire la complication des phenomènes de division."

Dangeard ('93) found that the nucleus elongated and the nucleolus divided in two by an elongation to a thread-like process within the nuclear wall. Swellengrebel ('05) and later Fuhrmann ('06) ascribed a definite mitosis to the nucleus, the latter studying *Saccharomyces ellipsoideus* I. Hansen and presenting a typical karyokinetic sequence with the formation of four chromosomes. There were several authors who held to this view, and at present there are those who believe that definite mitotic phenomena are present. Kater ('27), using a smear technique with *S. cerviciae* [sic], demonstrated a mitosis, with the formation of about eight chromosomes and a definite spindle, instead of division by constriction. It should be pointed out here that smear methods as applied to yeasts and particularly as carried out by Kater have never yielded any results which might be considered reliable. The act of smearing, no matter how good or how careful the application of the fixative might be, usually allows for some action on the cell wall that obliterates the correct phenomena and substitutes artifacts, so that vacuolar constituents or secretion products of the cytoplasm have been misinterpreted.

On the other hand, Guilliermond ('17), in a summary of his work, found amitosis to occur where budding was present. This process was characterized by an elongation of the nucleus which quickly divided by the resorption of the thread-like portion that separated the two segments. Guilliermond also observed that it was impossible to see the nuclear phenomenon clearly due to the abundant products of secretion which covered the nucleus. The observations of Kohl, of Wager and Peniston ('10), and of Pénau confirmed Guilliermond's results, and even went so far as to actually claim definite amitosis.

Whether or not mitosis or amitosis actually occurs is still a matter of dispute, despite the evidence that either side may advance. There are several factors which must be considered in studying the process, or processes, in nuclear division. First, does the nucleolus have a single morphological characterization, such as exists in the higher forms, or does it constitute the total nucleoplasmic material, as some call it, which has an affinity for iron haematoxylin, for example, as in pl. 22, fig. 18? Second,

can the nucleus be seen so clearly that there is no mistaking the change that may take place during the actual process? The first question is difficult to answer because it has been found by several workers studying the chemistry of the cell, yeast or yeast-like or even that of the higher Ascomycetes, that certain materials take the same dye. Then, the linin network with the chromatin material or the chromomeres, as some call them, usually cause confusion. The second question is really serious, because we know that our most precise methods of technique are still too crude for the minute nuclear make-up of these lower Ascomycetes. Cell substances, or secretion products as Guilliermond calls them, usually take stains which are peculiar to the nucleus and mask any clear-cut pictures of nuclear phenomena, and nucleic acid substances, derivatives of excretion products of the nucleus, probably nucleophosphates and the like, are also substances that must be considered in detail. Besides, the size of the reactors in the process is too small for any precise determinations with the means at hand.

On the other hand, according to the theories of modern genetics there must be some mechanism whereby chromatic substance or material is distributed through the agency of mitosis, whereas the act of amitosis is simply a means of increasing the nuclear surface or spreading the nuclear material through a cell and is comparable to the fragmentation or lobulation of nuclei. The latter is not at all reproductive in this respect. However, there are many acts taking place in the daily life of a fungus, particularly in these lower groups, which are impossible to explain with our present knowledge of cytology or genetics, and theories have been built up only to be torn down and built up again. This does not mean that genetics is not standing on firm ground, but that much more must be learned about mycological phenomena before any generalizations can be made.

It is difficult to understand that the nucleus in the hyphae of yeast-like organisms, *E. capsulatus* or *E. dermatitidis*, divides by direct division, at least as far as can be made out from the nuclear appearance. The process is evidently very rapid, for an examination of a great many slides failed to show any condition other than that shown in pl. 22, figs. 3–5. In the

ascogenous hyphae, or perhaps the antheridium and ascogonium (pl. 22, figs. 6–9), the division is somewhat slower, and here an elongation of the nucleus and nucleolus may be seen with a deeper staining central portion indefinite as to character but perhaps analogous to chromosomes.

In a discussion of nuclear phenomena, the next thing to be considered is the mode of development or reproduction. Guilliermond ('05, '05a, '05b, '08, '09, '09b, '10, '10a, '10b, '11, '11a, '12, '13, '17, '19, '20), Dangeard ('93, '94, '94a, '94b, '97), Hansen ('04), and several others, in a long series of investigations, have described the sexuality of the lower yeasts and some of the yeast-like fungi. It is known, of course, that in the Ascomycetes, particularly the lower forms, there may be three forms of development, heterogamy, isogamy, and a reduction to parthenogenesis. In the first case, two gametes, usually of unequal size, a small one generally representing the antheridial cell and a larger one the ascogonium (this is not a hard and fast rule), send out one, or sometimes several, small tubes which copulate and fuse. The cell contents of the antheridium then pass into that of the ascogonium. The two nuclei, one from each gamete, fuse, and finally an ascus develops through the subsequent division of the fusion nucleus, the presumptive mother spore. In the case of isogamy in the yeasts, two morphologically alike gametes fuse in like manner. Finally in the series, there is a reduction to a condition in which a cell may suddenly produce spores without copulation. This condition has been found by Mangenot ('19) for *E. Lindneri* and by Guilliermond for several other species, as *E. fibuliger* and *Zygosaccharomyces Pastori*. It occurs also when an ascogonial cell sending out a tube or tubes fails to copulate and hence produces spores parthenogenetically.

In some species the above phenomena may be found separately, or two processes or even all three may be present in the same culture. This latter condition has been found to be true for *E. capsulatus*.

In addition to the parthenogenetic formation of asci and ascospores, there is a non-sexual reproductive structure which has not been given too much attention in the past. This is the conidium (pl. 23, fig. 10), which is a non-nucleated structure

filled with chromatin granules or the metachromatic corpuscles emphasized by Guilliermond. It occurs usually near a septum of the hypha, and measures approximately 5 μ in diameter. Structures of this sort may and usually do spring up from the hyphae when there is a lack of nutrient material, or when the hydrogen-ion concentration is fairly high (pl. 19, figs. 4, 7–8, 11–12). These may be pyriform (pl. 18, fig. 10) or round (pl. 18, fig. 7), sessile or on a short pedicel, with a thick wall (pl. 23, fig. 10) or a thin wall. There is a heavy reticulated network with the granules mentioned above usually occurring at the nodes of the threads. These particular structures break off easily and may serve as resting cells or chlamydospores which, when placed in favorable media, germinate, form nuclei by an accumulation perhaps of the chromatin material, and develop a normal growth. They represent probably a degeneration from an ascus, or, on the other hand, an advanced character of reproduction.

To get a clearer understanding of the life history of *E. capsulatus*, it would be advisable to begin with the organism as it is found in the parasitized host and explain the mechanism by which it and related fungi propagate themselves.

In the tissue the organism grows as a yeast 6–8 μ in diameter, and reproduces itself by budding. There is no nucleus present, at least it could not be demonstrated in tissue sections or in freshly isolated cells, but instead there is a distribution of nuclein material throughout the organism, much the same condition as exists in bacteria, Myxophyceae, or in the conidium shown here. When isolated and grown on an artificial substrate as on agar, the chromatin or nuclein material seems to become larger, and there is usually formed a nucleus and many large granules which may be presumptive nuclei. This latter condition is based on circumstantial evidence (pl. 21, figs. 4–9; pl. 20B). The cells then pass through this stage, the yeast-cells, to stage two which consists of large irregular cells, attaining a condition as seen in pl. 19, figs. 14–15, 20–25, and have been clearly shown for *E. dermatitidis*. At this stage the chromatin material is spread throughout the cell, but the reticulated network seen in the later stages is not very clearly established. However, it seems to be rapidly developing and nuclei are clearly distinct. The stage

following is probably the most complex of all, inasmuch as there is a diversity in morphology and cytology, changes due probably to hydrogen-ion concentration and temperature and several other factors among which the changed habitat is outstanding. In this third and final stage, we find that sexuality has developed and asci with ascospores are produced. To follow the life cycle on an artificial substrate, it would then seem best to follow the development from the germinating ascospore. In doing this, it is necessary to consider first normal growth on some favorable medium, and second to correlate the nuclear changes in material fixed in hanging-drop preparations (Van Tieghem cells) and stained, with the nuclear phenomenon found in the material fixed and stained as outlined in the technique. It was noticed that from three to fifteen days were necessary for the life cycle of the fungus to be completed, depending on the broth used in the hanging-drop preparation.[2] It was found that proteose peptone broth gave good growth as did lactose broth, but that bacto-peptone plus bacto-beef gave quickest development of asci, from three to five days usually. The material was fixed by the addition of two or three drops of a fixative described previously, and after a period of from six to eight hours, or over night, the fluid was drawn off with filter-paper and the material was carefully washed with distilled water and then mounted in lacto-phenol plus crystal violet, or stained with iron haematoxylin. By keeping several of these hanging-drop cultures growing, it was possible to obtain different stages of growth, even in one preparation. The method was of course not entirely accurate cytologically, but it served the purpose as demonstrated here.

The single spore (2–2½ μ) is found to be made up of densely staining granular material, but the granules are so small that it is difficult to discern them. On germinating, a tube is sent out or the spore simply elongates, taking with it the chromatin material (pl. 22, figs. 1–2). When somewhat older, the developing hyphae produce nuclei which divide rapidly as pointed

[2] Several solutions were used: lactose broth (product of Digestive Ferments Co.), pH 6.8; 2 per cent bacto-peptone broth, adjusted to pH 7.0; 2 per cent proteose peptone broth, pH 7.0; bacto-peptone plus 6 per cent glycerine; meat extract broth, pH 7.1; 2 per cent bacto-peptone plus 5 per cent bacto-beef (dehydrated), pH 7.2.

out previously, are distributed in pairs very close together, and then separate (figs. 3–4). At this time the reticulum is well developed and the chromatin granules, or basophilic grains, as Guilliermond calls them and which he believes probably represent albuminoid bodies playing the rôle of products of nutrition in the form of perhaps zymogen or reserve material, are very evident and take a deep stain with haematoxylin. At the same time, the wall increases in thickness and becomes fairly evident as in fig. 4. The number of nuclei in the hyphae varies, as many as thirty-seven having been counted on one hypha, but this was apparently rare for the amount usually varied between seven and fifteen with nine the most common, as in fig. 4. With the elongation of the new hypha, septa are laid down (fig. 5) and the formation of conidia as described previously (pl. 22, fig. 5; pl. 19, fig. 4) may be and usually is associated with this act. Following this, the hyphae, varying from 1½ to 4 μ on different media, may grow out to form branches or reproductive structures, usually lateral or terminal. However, on acid media or on media where there is a definite lack of some suitable protein or protein product in the form of peptone, peptides, or amino acids, there is an excessive formation of chlamydospores, either terminal (hypnospores), 4½–6 x 9–12 μ, or lateral, 5 x 7 μ, or intercalary, approximately 5 μ in diameter, and conidia (pl. 18, fig. 7; pl. 19, figs. 7, 11–12). With the apparent development of a hypha, there is an accompanying formation of sexual cells. This process may be slow or rapid, depending on conditions. On glycerine agar it seemed to be the most rapid, these particular organs being produced in three to five days. The antheridial or male cell contains two or three nuclei as a rule (pl. 22, figs. 6–8), but cases of four have also been seen. The ascogonium or female cell usually contains three to four nuclei (figs. 9–11), but five (fig. 12) have also been noticed.

When two sexual cells are ready for copulation, the antheridium bends over to meet the ascogonium as seen in pl. 22, figs. 10–11. The two gametes may be on the same hypha or on separate hyphae. Both may be terminal (figs. 13–17), or both lateral (fig. 16), or one may be lateral and the other terminal (fig. 12), or there may even be variations of these. Nevertheless it is

quite apparent that the male cell goes to meet the female cell. With the approach of the copulation branches, there is an action which may be termed tropistic and which may be explained, if analogies are acceptable, by a hormone action or physical stimulus as is found in zoological specimens, which calls forth the production of a beak, the copulating tube, as has been found in the lower yeasts. These two beaks meet and fuse. In the meantime, one of the nuclei proceeds to the tip of the beak. There is probably no specific nucleus involved in this act, because as far as can be made out all the nuclei are the same morphologically, and any nucleus that happens to be nearest the beak becomes sensitized to the fertilization reaction. With the progression of the fertilization nucleus, there is an accompanying retrogression of the remaining nuclei. Whether these disintegrate or not has not been definitely determined. In several cases where fertilization had taken place, it was noticed that the antheridial cell and basal portion of the ascogonium merely existed very much like functionless stamens in a flowering plant, which in the course of time disintegrated or perhaps degenerated.

The next step in the cycle involves the fusion of the nuclei, or syngamy perhaps. The walls between the two tubes are dissolved or diffuse and the two nuclei copulate or fuse in the tube (pl. 22, figs. 13–16). This is rather contradictory to Dangeard's ('94, '94a, '94b, '97) observations that the two nuclei fused in the ascus, and Harper's ('96, '97, '99, '00), that in the higher Ascomycetes the fusion occurred in the archicarp after the entrance of the contents of the antheridium. The fusion nucleus is then transferred or migrates (whatever the process may be it is analogous to that by which the nuclei copulate) into the ascogonium which is now known as the archicarp (pl. 22, fig. 17). The fusion tube dissolves in some cases or breaks away in others, with the broken portion being either resorbed by the cells or degenerating. At any rate, the archicarp repairs its walls by the resorption of the cellular contents, and the large fusion nucleus is ready for division (fig. 18).

The nucleus divides by a process of amitosis as previously mentioned. Although difficult to interpret theoretically, it presents no clear-cut chromosomal formation. With the first

division, there is apparently the formation of a wall (pl. 22, fig. 19). The daughter nuclei go through a synchronous division (fig. 20) to form four nuclei. In several of the yeasts and in *E. Magnusii*, the process halts here and each nucleus develops into a spore, thus four spores. Here, however, the four nuclei undergo a synchronous division, the second for the ascus, and eight nuclei are produced, which number is a mean for the species of this genus. In other species there may be twelve or sixteen nuclei.

In the higher Ascomycetes the problem of ascus formation is rather confusing, and the reader is referred to the work of Atkinson ('15), Bagchee ('25), Blackman and Fraser ('05), Brooks ('10), Brown ('10, '11), Carruthers ('11), Guilliermond, Mangenot and Plantefol ('33), Faull ('05), Fraser and Brooks ('09), Fraser and Welsford ('08), Gaümann and Dodge ('28), Harper ('96, '97, '99, '00), and Maire ('04, '05), to obtain full information and various opinions on the subject.

After the formation of the nuclei and the abjunction of the future ascus (8–14 μ in diameter) from the basal portion of the ascogonium, now a basal cell as seen in pl. 22, figs. 21–22, the development of the spores begins. Associated with this phenomenon are the secretion products which, as has been pointed out previously by Guilliermond, nourish the spores and produce the spore wall.

It should be pointed out here that, in addition to this heterogamous form of copulation, isogamy and finally parthenogenesis may also be present. The latter is quite apparent and has been found time and time again. The process may begin by the jutting out of what is apparently a conidium. This in turn develops and a nucleus becomes visible. The nucleus divides as noted in the above reaction and an ascus with eight ascospores forms (pl. 23, figs. 1–9). Also a terminal cell often produces a pyriform ascus parthenogenetically as seen in fig. 11. In all cases, the spores serve the same function and are equally as capable of producing new hyphae. The sporogenous plasma of the ascus, part of the cytoplasm, contributes greatly to the formation of the mature spore.

The life cycle of *E. capsulatus* on an artificial substrate might be graphically illustrated as in diagram 1.

Cellular contents.—The remainder of the ascus constitutes the epiplasm which is made up of reserve products in the form of lipoids, glycogen, the metachromatic granules of Guilliermond or nuclear decomposition products as volutin, oil globules, and nucleic acid substances and probably other protein derivatives and carbohydrates. All of these materials have been shown to be utilized by the spores in their growth. Several authors also present evidence that during the maturation process of the spores,

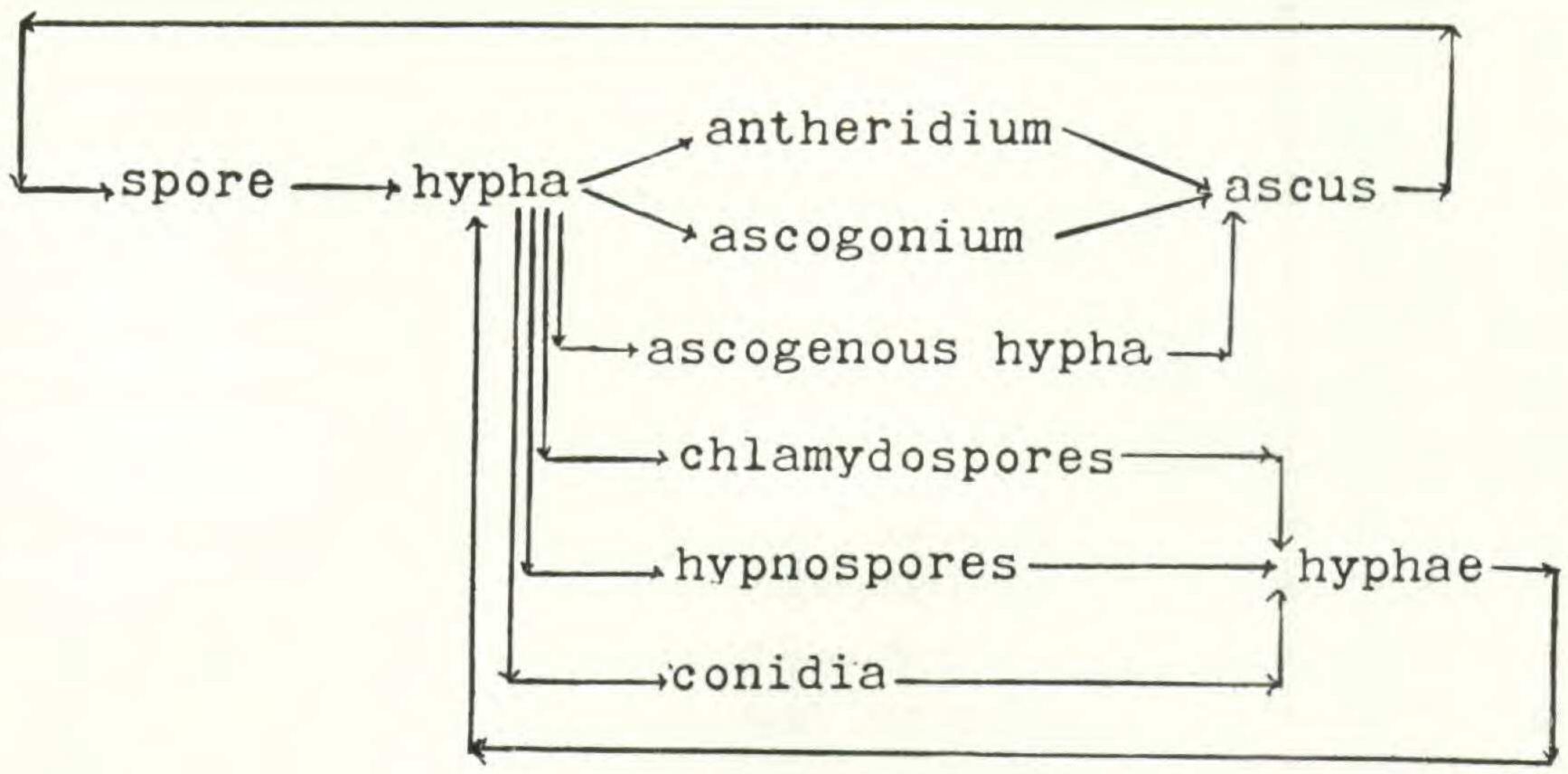


Diagram 1.

the substances constituting the epiplasm are broken down, a part being absorbed and clearly demonstrable in the granular contents at maturity, and a part reserved for their germination.

Volutin.—The cellular contents will be considered in the order studied. The first is what has been variously called "Neisser's granules" found by Neisser in bacteria; "sporogenous grains" found by Ernst; "metachromatic corpuscles" of Babès, who found them in the diphtheria bacillus and so named them because of their metachromatic action; "red grains" of Bütschli, so named because they took on a red coloration with many of the stains; and finally, one of the most common of all, volutin, as named by Meyer.

In the fresh state these granules stain blue with methylene blue (pl. 23, figs. 16–18), and when fixed their color is red to violet. They are small at first, and then enlarge to assume various forms and sizes. They may have a heavy staining outer portion and

a paler center, probably due to their refractive powers. They are seen quite often in vacuoles as represented here and assume fantastic features. They appear in the cell along the reticulated network, at the nodes, and stain heavily there. Volutin is very common in the younger cells, but not so abundant in the older hyphae. The substance may also be stained *in vivo* with neutral red. Wager and Peniston ('10) found Gram's aniline violet to be a useful stain for volutin.

Zikes ('22), studying the nature of volutin and of the physiological factors concerned in its production, found that it is present in moderately large amounts in nearly all fungi. Peptone when added to the culture media stimulated its production while ammonium sulphate and asparagin were not so favorable. Phosphorus was found to be essential for its formation, and glucose and fructose were more favorable than the carbohydrates of higher molecular weight. He concluded that volutin is an albuminous substance similar to the nucleo protein, as it contains both phosphoric acid and nuclein.

The general opinion seems to be that volutin is a nucleic acid substance with an unknown base, probably organic in nature, that it exists as a colloid, and that, due to fixatives, it is precipitated in the vacuoles. It is supposedly a secretion from the nucleus and is used in nourishing the spores.

Glycogen.—Another substance which supposedly acts as a reserve material and is used up for the maturation of the spores in the ascus is glycogen. This theory has been substantiated by the fact that glycogen is used up in the cell, particularly in maturing asci, as evidenced by its slow disappearance. Kohl ('07) considered glycogen not as a reserve material, but as a regulator for the intake of sugar in the cell, in that it is the substance formed, not being able to diffuse out from the membrane. He found it to be lacking in spores, and since reserve materials should be present, he presented this as evidence that glycogen is not a reserve material. Guilliermond, however, claims to have demonstrated that glycogen is absorbed by the ascospores at the time it is found to disappear from the epiplasm.

Glycogen is found in the organism practically from the beginning of growth, first as small droplets perhaps, but later in

fairly large masses (pl. 23, figs. 19–30). It has been estimated as constituting 32 per cent of the dry weight of yeast cells. It is associated with the nutritive condition by some workers and found to be almost entirely used up or greatly accumulated according to the condition of nutrition and growth. According to some workers it appears somewhat in the form of a vacuole and it has thus been called a glycogen vacuole. It is not a true vacuole, but perhaps a colloidal substance, as volutin is considered to be, for it takes a stain much as the latter does. It may be stained *in vivo* with neutral red (figs. 26, 29–30), in which case it is not so clear, at least in *E. capsulatus*, as with a saturated solution of iodine potassium iodide, a modification of Gram's stain (figs. 19–25, 27–28).

This substance is a glucoside, a polysaccharide which, like starch, is made up of n molecules of glucose to have the formula $(C_6H_{10}O_5)_n$. It is often compared with starch since it requires phosphorus for its formation. In fact, it has often been referred to as an amylopectin, which makes up the superficial part of the starch grain. By hydrolysis, glycogen is broken down to dextrin, then maltose, and finally glucose. On heating a saturated solution of glycogen stained with iodine potassium iodide it becomes pale but regains its intensity on cooling. It is insoluble in alcohol.

Vacuoles.—The question of vacuoles in fungi has been given considerable attention in the past, and the author does not intend to enter into a discussion of them. However, in view of the many theories advanced as to their character and presence, mention of them in *E. capsulatus* would not be out of place. They are easily demonstrable with many dyes and present varied characteristics. With methylene blue, they are easily made visible (pl. 23, figs. 15, 17–18). They appear to be formed by the reticulum and to take the stain. They may be demonstrated fairly clearly with iodine green (fig. 14), and the standard dye seems to be neutral red, in which case small crystalloid bodies, often described as "dancing bodies," probably Brownian movement, may be seen (figs. 26, 28). Haematoxylin also brings them out distinctly by staining the surrounding network (figs. 1–2). What the function of vacuoles may be is difficult

to understand. It is known that they are colloidal solutions of cytoplasmic materials, formed by the absorption of water, that they may enlarge or disappear in a cell, and Guilliermond, Mangenot and Plantefol, say this of them, "Les vacuoles représentent la phase aqueuse du cytoplasme et jouent certainement le rôle important en réglant la teneur en eau de la cellule."

Chondriosomes.—The bodies designated as mitochondria or chondriosomes have been demonstrated in animal and plant tissue. What their particular function may be is not definitely known. In plants they have been associated with the elaboration of chlorophyll and starch through the agency of the chloroplasts. In animals they are supposedly associated with the elaboration of secretions, but no definite evidence has been produced along this line. They have been demonstrated in a number of lower plants, lower fungi and the higher Ascomycetes, and in fact, in practically all of the groups, as well as in animal cells. For further information the reader is referred to Guilliermond ('11b), who has traced the development of these particular bodies in many organisms.

Chondriosomes (Cowdry, '17) have been shown in both animal and plant cells to be almost completely soluble in alcohol, ether, chloroform, and dilute acetic acid (organic solvents) and to be insoluble by chromization and treatment with formalin, at least in most cases. In the animal cell they do not stain with Sudan III or IV and are sometimes blackened with osmic acid. In plants, the whole cell blackens with osmic acid so readily that it is impossible to know just to what extent the chondriosomes themselves are affected.

In studying *E. capsulatus*, the author made use of the knowledge that chondriosomes are very clearly demonstrated by the addition of iodine potassium iodide to a preparation of an organism. They are fairly evident as lightly staining rod-like and short bodies (light yellow with iodine), but one must pay particular attention to distinguish them (pl. 23, figs. 19–25). There are short and rod-like bodies in the terminal hypnospores and some long forms too, as well as small point-like reflective granules. The rods are longer in the hyphae (figs. 23–24). Chondriosomes are scattered throughout the asci as fine, small, rounded bodies.

It can thus be stated that by the iodine potassium iodide method, bodies comparable to those described as chondriosomes for other fungi are demonstrable.

Fat, lipoidal substances.—In addition to the many substances mentioned, there are also fats, lipoidal substances, and other materials called reserve materials, secretion products, and also excretion products. These materials are found in varying amounts in many of the specialized portions of the organism, being abundant in the asci and chlamydospores and in very small amounts in young hyphae and younger elements. They can be demonstrated comparatively easily in spores and in yeasts particularly. The nature of many of these substances has not been determined as yet, but it is generally known that fatty acids, glycerides and sterides, glycerol, phospholipides and phosphoaminolipides (complex lipides) may be present in certain amounts.

Several agents were used for the study of these materials, each giving some degree of difference in distinguishing them. A 2 per cent osmic acid solution which reduces fats and gives them a black coloration was the first tried. When applied to the living mycelium, the fats and lipoidal substances appear as small refractile bodies or droplets but in some cases they are very much larger (pl. 23, figs. 31–36). They are seen rather abundantly in old hyphae (fig. 36) and in very small amounts in young hyphal tips (fig. 35). With platinic chloride (5 per cent solution) they appear as blackened granules much the same as with osmic acid (figs. 37–38). Iodine potassium iodide as applied for glycogen and chondriosomes shows lipoidal substances, as oil droplets, equally as well. These are very small, highly refractile, and hyaline, and to be seen require careful focusing and adjustment of the microscope (figs. 19–20, 23–25). Neutral red has also been used, but not much attention was paid to the reaction outside of what has already been mentioned with regard to glycogen and the vacuoles. Iodine green also has an affinity for lipoidal substances, but not enough attention was given to it to prove its value here.

CULTURAL CHARACTERISTICS

In studying the cultural characteristics of *E. capsulatus*, all the media available at the time were utilized. This was considered essential, inasmuch as it has become an unfortunate custom with some mycologists to name as new species organisms which show a physiological variation on a different medium. Since the fungus had shown on previous occasions of culturing that it favored protein substrates, a variety of protein materials was chosen. In addition, regular routine media were used, some with much carbohydrate and some with very little. For some years the question of hydrogen-ion concentration has become an important factor in work of this sort, and media with a wide range of pH were chosen within which the possibilities of growth had at one time or another been emphasized. All cultures were grown at a temperature of approximately 25° C.

The following media arranged in the order of their decreasing hydrogen-ion concentrations were used:

Raulin's Solution Agar (*Raulin's solution plus 1.5 per cent agar, pH 4.1*).—(pl. 18, fig. 7; pl. 19, figs. 8, 10–12). Growth poor, being present only around inoculum after 18 days and having a diameter of only 0.7 cm. and 1.6 cm. at end of 30 days. Color white. Hyphae grown heaped up from center in a loose fashion. Microscopically, abundance of conidia, pedicelled or sessile, 5 μ in diameter; endo-chlamydospores 5–7 μ in diameter; terminal hypnospores 4–6 x 8–11 μ; hyphae 2 μ in diameter; racquet mycelium in small amount, not very noticeable; asci 9–10 μ in diameter.

Richards' Solution Agar (*Richards' solution plus 1.5 per cent agar, pH 4.3*).—(pl. 19, figs. 5–7, 9). Growth slow, very sparse and cottony, with long and narrow hyphae 2 μ in diameter, projecting loosely and irregularly from the edge of the colony which attained a diameter of approximately 1.8 cm. at end of 18 days and 3.5 cm. at end of 30 days. Round conidia very numerous, approximately 4 μ in diameter; pyriform conidia sessile or on short stalks, several, 3½ x 7 μ in diameter; round, thick-walled chlamydospores on short pedicels, 5 μ in diameter; asci few, 7–9 μ in diameter; endo-chlamydospores approximately 6 μ in diameter.

Czapek's Agar (pH 4.4).—Growth of very loose and sparse mycelium which spreads over the surface of the agar with thin hyphae. Macroscopically the culture is barely visible except by reflected light. Colony approximately 3 cm. in diameter at end of 18 days and 6.7 cm. at end of 30 days. Color white. Hyphae 2½–3½ μ in diameter; racquet mycelium present, 6–7 μ in diameter at swollen portion and 2½–3 μ at thin portion; terminal hypnospores 7 x 12 μ approximately; conidia many, 5 μ in diameter; round chlamydospores 6 μ in diameter; lateral chlamydospores 6 x 11 μ; asci several, 10 μ in diameter.

Wort Agar (Product of Digestive Ferments Co., pH 4.6).—(pl. 18, figs. 6, 8–10, 15). Growth at first slow, none on several of the cultures, in general thin and loose. One of the cultures showed good growth, attaining a diameter of 3.2 cm. at end of 18 days and 5 cm. at end of 30 days. This was unusual and may be ascribed to too great an inoculation and to a dissemination of spores as a result of shaking. Color white. Terminal hypnospores many, 7 x 12 μ; hyphae 3½–4 μ in diameter; chlamydospores 6½–7 μ in diameter; racquet mycelium abundant; conidia numerous, 5–6 μ in diameter; asci 10–11 μ in diameter.

Malt Extract Agar (pH 5.1).—(pl. 18, figs. 1–3). Growth not so abundant, colony 1.6 cm. at end of 30 days. Color slightly brown, due to the malt extract. Mycelium of numerous swelled cells; hyphae 3–4 μ in diameter; terminal hypnospores 6 x 16 μ; hyphal swellings or chlamydospores 9 x 12 μ; numerous conidia, pyriform and round, the round being approximately 5 μ in diameter; asci very few, 10 μ in diameter.

Malt Extract Broth (The above minus the agar).—Very little growth at end of 30 days. Characteristics same as above.

Maltose Agar (pH 5.4).—Growth slow. Colony coremium-like, with a diameter of 1.5 cm. at end of 30 days. Hyphae short, 3–4 μ in diameter. Many round chlamydospores 5 μ in diameter; terminal hypnospores numerous, 4–6 x 9–11 μ; asci few, 8–10 μ in diameter.

Sabouraud's Broth (Sabouraud's dextrose agar minus the agar, pH 5.5).—(pl. 21, fig. 21). Culture consists of submerged colonies of mycelium varying from ½ to 3 cm. in diameter at end of 18 days, with a great mass at the end of 30 days as a result of the

coalescence of all the colonies. The large flakes are grey in color when moist, but with white mycelium on the surface, fairly dry. Submerged hyphae 2½ μ in diameter with none or very few morphological characteristics. Aerial or dry mycelium, however, similar to that on the agar culture of the same medium.

Sabouraud's Agar (pH 5.6).—(pl. 16, figs. 12–14; pl. 19, figs. 13–25; pl. 21, figs. 14, 18, 28). Thick cream-colored growth with a diameter of 4.5 cm. in 18 days and 7 cm. in 30 days. Hyphae 2–3½ μ in diameter, in needle-like projections from the surface of the mycelium which appears very cottony. Color white. Older cultures show a felt-like matting with a tendency towards ridge formation, a condition found to a certain extent in *Microsporon Audouini* Ota and Langeron. Racquet mycelium present with swollen portions 4–6 μ and narrow section 3 μ; many lateral chlamydospores, 5–7 x 10–12 μ; terminal hypnospores 5 x 11 μ; and many conidia, pyriform or round, approximately 5 μ in diameter; numerous asci, 10–12 μ in diameter, with 8 ascospores 2–2½ μ in diameter.

Oat-Meal Agar (Decoction of oat-meal plus dextrose and agar, pH 5.9).—Growth diffuse and slow, 5 cm. in diameter after 30 days. Culture loose and cottony. Hyphae 2–2½ μ in diameter; numerous thick-walled, round resting cells or chlamydospores, 4–6 μ in diameter; terminal hypnospores 4 x 7 μ; conidia numerous, 4 μ in diameter; asci several, approximately 8 μ in diameter.

Corn-Meal Agar (Product of Digestive Ferments Co., pH 6.0).—(pl. 16, figs. 8–9; pl. 21, fig. 24). Growth very loose and thin, with the hyphae projecting from the colony, appearing as threads of silk. Colony barely visible except from a lateral view. Hyphae long and thin, 2–2½ μ in diameter, with colony attaining a diameter of 5 cm. at end of 21 days and 6 cm. at end of 30 days; chlamydospores 6 μ in diameter; conidia many, 4½–5 μ in diameter; terminal hypnospores 5 x 7 μ; racquet mycelium present but reduced in size, 3–4 μ at swollen portion; asci several, 7–8 μ in diameter.

Potato-Dextrose Agar (Decoction of potatoes plus dextrose and agar, pH 6.2).—(pl. 21, fig. 22). Colony 3.6 cm. in diameter at end of 30 days, edge smooth and round, no striations or other cultural changes as ridges. Growth thick and cottony at in-

oculum, with a thin periphery about 2–3 mm. in width. Color white. Hyphae 2–2½ μ in diameter and long; conidia numerous, 5 μ in diameter; terminal hypnospores few, 4 x 7 μ; chlamydospores rare; asci few, 8–10 μ in diameter.

Two Per Cent Aqueous Bacto-Peptone (Hanging-drop culture, pH 6.2).—(pl. 21, figs. 1–8, 25, 32). Mass of thick-walled hyphae ramifying and branching, 2–2½ μ in diameter; racquet mycelium not evident; numerous chlamydospores, 4 μ in diameter; asci 4–6 μ in diameter; few terminal hypnospores, 3.5–5 x 6–7 μ.

Two Per Cent Aqueous Bacto-Peptone Plus Five Per Cent Meat Extract (Hanging-drop culture, pH 6.2).—(pl. 19, figs. 1–4; pl. 21, figs. 9–13). Growth profuse, covering the drop in 4 days. Hyphae 2–2½ μ in diameter, with an abundance of racquet mycelium intertwining and branching; thick-walled cells, chlamydospores in abundance, as well as asci 10 μ in diameter.

Two Per Cent Proteose Peptone Plus Six Per Cent Glycerine (Hanging-drop culture, pH 6.2).—(pl. 16, fig. 7; pl. 17; pl. 21, figs. 15, 30). Growth similar to that on bacto-peptone broth, but with an abundance of chlamydospores and racquet mycelium.

Lactose Broth (Product of Digestive Ferments Co., pH 6.8).—(pl. 21, fig. 17). Growth of submerged large flakes of colorless mycelium measuring approximately 2 cm. in diameter at end of 18 days. These later coalesced or intertwined into a mat which grew up the sides of the flask to form a white mycelium. Submerged hyphae approximately 3 μ in diameter, branching, intertwining, with cross-walls. Swellings, chlamydospores, terminal hypnospores, and asci few in number and reduced in size as compared with those on agar. The aerial mycelium above the surface of the broth showed an increased number of characteristics which simulated those found on the agar.

Lactose Agar (The above medium plus 1.5 per cent agar, pH, 6.8).—(pl. 18, fig. 11; pl. 19, fig. 20). Growth rapid, thick and cottony, colony attaining a diameter of 3.6 cm. at end of 18 days and 6.2 cm. at end of 30 days. Culture showed 4 ridges radiating from a thick inoculum to a thick cottony circular periphery surrounded by a thin growing rim of hyphae. Culture similar to that on nutrient agar, macroscopically. Hyphae 2½–3½ μ in diameter; conidia many, 4½–5 μ in diameter; terminal hypno-

spores 4 x 7½ μ; racquet mycelium present in abundance; many chlamydospores, varying from 6 μ in diameter (round) to 5 x 8 μ (pyriform); asci many, 8–9 μ in diameter.

Nutrient Agar (Product of Digestive Ferments Co., pH 6.8).—(pl. 21, fig. 19). Growth good, with the colony having a diameter of 3.5 cm. at the end of 20 days and 7.0 cm. after 30 days. Growth loose and cottony, showing concentric circles, evidently due to a periodical formation of asci and the liberation of the spores which germinated. Single spore colonies 2 cm. in diameter after 15 days. Colony flat with age, and white. Hyphae 2–3 μ in diameter; conidia many, sessile or pedicellate, 5 μ in diameter; chlamydospores several, 6 μ in diameter when intercalary, 5 x 7 μ when lateral, 6 x 11 μ when terminal (hypnospores); racquet mycelium present, 5 x 3 μ.

Nutrient Broth (Meat extract, pH 6.8).—(pl. 21, fig. 31). Culture similar to that on lactose broth.

Uschinsky's Protein-free Medium (pH 6.8).—No growth.

Beef Extract Agar (Liebig's extract of beef, pH 7.0).—(pl. 16, fig. 15). Growth similar to that on lactose agar. Radiating ridges present.

Eosine-Methylene-Blue Agar (Product of Digestive Ferments Co., pH 7.0).—(pl. 21, fig. 27). Growth good, attaining a diameter of 5 cm. after 26 days. Color pink in younger portion of culture (periphery), darker pink to blue towards the inoculum, this being due to an absorption of the dyes by the mycelium. Growth ceased and colony became flat. Hyphae in growing culture 2–3½ μ in diameter, with morphological characteristics similar to those on Sabouraud's agar.

Glycerine Agar (Nutrient agar plus 6 per cent glycerine, pH 7.1).—(pl. 16, figs. 1–3, 11; pl. 21, figs. 16, 23, 26, 29). The colony on this medium grew as a cerebriform, very thick, creamy culture, having a diameter of 6 cm. at the end of 30 days. Medium best for study of organism because of its nutrient constituents, presence of abundant protein and carbohydrate, and an abundance of endo-chlamydospores, 5½–6 x 11 μ; intercalary chlamydospores 4–5 x 5–7 μ; terminal hypnospores 4–6 x 9–12 μ; hyphae 2½–4 μ in diameter; round chlamydospores, either terminal or lateral, approximately 6 μ in diameter; racquet mycelium abundant; asci numerous, 10–14 μ in diameter.

Blood Agar (pH 7.2).—(pl. 16, figs. 4–6). Growth similar to that on nutrient agar.

Chocolate Agar (Blood agar heated to about 75° C. until the blood became chocolate colored, pH 7.2).—Growth similar to the above.

Serum Agar (Beef extract agar plus 10 per cent dehydrated blood serum, pH 7.2).—(pl. 18, figs. 4–5, 12–14, 16). Growth moist, showing a diameter of 1.4 cm. at end of 10 days and 3.5 cm. at end of 30 days. Colony flat and even. Many yeast-like cells seen; hyphae few and several large cells. Center of colony cerebriform. With age the culture shows prickly forms of mycelium (dry) which covers the greater part of the culture. This evidently is a reversion to the yeast form, to a certain extent.

Calcium Carbonate Agar (pH 7.4).—(pl. 16, fig. 10). Growth slow at first, then rapid, with a diameter of 7.4 cm. in 30 days. Colony compact, with a cerebriform central portion, and a loose, cottony outer zone. Color white. Hyphae 2–2½ μ in diameter; conidia numerous, 4½–5 μ in diameter, being spread throughout the entire culture, mostly round, several pyriform on short peduncles, others sessile; round chlamydospores many, 6 μ in diameter, and terminal hypnospores 4–5 x 7–9 μ; racquet mycelium not very evident; asci many, approximately 8 μ in diameter.

Endo's Agar (Product of Digestive Ferments Co., pH 7.5).—Growth slow, not quite so rapid as that on eosine-methylene-blue agar. Colony white at first, then, due to an absorption of the dye from the agar, pink to red, growing in coremium-like masses of straight hyphae with very few or no conidia. Hyphae short and thick-walled, 3½–4 μ in diameter. Few conidia in culture, 6 μ in diameter; hypnospores very few, as well as round chlamydospores; asci several, 10 μ in diameter.

Litmus Milk.—Milk was heated in a flask for fifteen minutes in steam, then set away over night in the ice-chest to allow the cream to rise. The cream was then siphoned off and the milk diluted in the ratio of 1 part milk to 4 parts water, with enough litmus as an indicator. Tubes of this solution were sterilized by steam, then inoculated, and kept at 25° C. No growth resulted after 20 days.

Reaction to Temperature.—In studying an organism, it is necessary, especially if quantitative results are desired, to determine conditions under which optimum growth can be obtained. One factor which enters into such considerations with fungi is temperature. It is known that various organisms grow best at certain temperatures and when placed in other conditions, growth will either be inhibited or retarded. With this point in mind, it was decided to grow a number of cultures on the same medium and same pH at various temperatures. As a medium, Liebig's beef extract agar at pH 7.2, which on previous occasions had shown a qualitatively and quantitatively good growth, was employed.

E. capsulatus, when first isolated from the lesions in its yeast-like form, showed very good growth at body temperature, 37.5° C. After having been kept in culture in its filamentous form for about two years, the optimum temperature was shown to be approximately 30° C. Continued growth on an artificial medium again reduced its optimum temperature, to approximately 25° C., as may be seen in table 1, or figs. 1–2.

Several cultures in petri dishes were grown at the various temperatures indicated in table 1. The experiment was performed at three different times, and due to the fact that daily measurements required handling of the cultures, several of the plates showed contamination. A procedure such as this usually brings in some foreign spores, especially in a large laboratory where many open cultures may be found.

The plates of the first two temperatures, −0.7° C. and 8.0° C., were kept in a Kelvinator, the former in the freezing unit where a fairly constant temperature was maintained, and the latter in a compartment some distance from the cooling device. The third set of plates, 16.0° C., was kept in a glass jar with running water around it. The temperature fluctuated somewhat, but not enough to affect the experimental data seriously. The plates of the other temperatures were kept in regulated incubators.

All the plates were inoculated with a loopful of a suspension mashed up with a sterile needle and contained 5 cc. of a beef extract broth solution of pH 7.2. In some cases the inoculum proved greater than others.

TABLE I

MEAN DIAMETERS IN CENTIMETERS OF COLONIES GROWN AT VARIOUS TEMPERATURES

Beef extract agar (Liebig's extract of beef) pH 7.2

Days after inoculation	Temperature in degrees Centigrade							
	−0.7	8.0	16.0	21.0	25.0	31.0	37.0	40.0
1	0.4	0.3	0.3	0.3	0.4	0.4	0.4	0.3
2	0*	0	0.3	0.4	0.5	0.4	0.4	0
3	0	0	0.3	0.4	0.8	0.7	0.6	0
4	0	0	0.3	0.5	1.0	0.9	0.7	0
5	0	0	0.4	0.7	1.3	1.1	0.9	0
6	0	0	0.6	0.9	1.6	1.3	1.1	0
7	0	0	0.8	1.1	2.0	1.6	1.3	0
8	0	0	0.9	1.4	2.2	1.8	1.4	0
9	0	0	1.1	1.7	2.5	2.0	1.5	0
10	0	0	1.3	1.9	2.7	2.2	1.5	0
11	0	0	1.4	2.2	3.0	2.5	0	0
12	0	0	1.6	2.4	3.3	2.7	0	0
13	0	0	1.8	2.6	3.5	2.9	0	0
14	0	0	2.0	2.8	3.8	3.2	0	0
15	0	0	2.1	3.0	4.1	3.4	0	0
16	0	0	2.3	3.2	4.4	3.6	0	0
17	0	0	2.5	3.4	4.7	3.9	0	0
18	0	0	2.7	3.7	5.0	4.1	0	0
19	0	0	2.9	4.0	5.2	4.4	0	0
20	0	0	3.1	4.2	5.5	4.7	0	0
21	0	0.3	3.3	4.5	5.7	4.9	0	0
22	0	0.3	3.5	4.8	5.9	5.1	0	0
23	0	0.4	3.6	5.0	6.2	5.3	0	0
24	0	0.5	3.7	5.2	6.5	5.5	0	0
25	0	0.5	3.8	5.5	6.8	5.7	0	0
26	0	0.6	3.9	5.6	7.1	5.8	0	0
27	0	0.8	3.9	5.7	7.4	5.9	0	0
28	0	0.9	3.9	6.0	7.7	6.0	0	0
29	0	1.0	3.9	6.2	7.9	6.1	0	0
30	0	1.1	3.9	6.5	8.2	6.2	0	0
31	0	1.2	3.9	6.7	8.3	6.2	0	0

*0 indicates no growth

Readings were made daily for 31 days, at approximately the same hour. The mean results of a representative series are given in table 1. Three plates were kept in the final table as representive of the experiment although several additional plates were used.

An analysis of the results of this experiment shows that no growth takes place at −0.7° C. and at 40.0° C. At 8.0° C. growth does not begin until the twenty-third day and then proceeds slowly, with a maximum diameter of 1.2 cm. which would represent a total growth of 0.9 cm. At 16.0° C. the culture does not show growth until the fourth day, and grows slowly but faster than that at 8.0° C., to show a final diameter of 3.9 cm. or 3.6 cm. actual growth. At 21.0° C. there is a final diameter of 6.7 cm., with a total growth of 6.4 cm. At 25.0° C. the diameter of the colony reached an optimum of 8.3 cm. and an actual growth of 7.9 cm., and at 31.0° C. the diameter of the colony was 6.2 cm. with a total growth of 5.8 cm. The culture at 37.0° C. grew fairly well for the first ten days but after that no further growth. Transfers from these last plates to fresh medium produced no growth, hence it could be concluded that the heat finally killed the organism.

In fig. 1, the diameter of the colony in cm. was plotted against the time in days to obtain the rate of growth for each temperature. It may be seen here how the growth was affected. At optimum temperature, after the initial lag period of 1–2 days, growth proceeded in practically a straight line. With a deviation from the optimum temperature there is accordingly a decrease in the growth rate which corresponds to the temperature affecting it. At 16.0° C. we find a regular sigmoid curve which represents an initial lag period followed by a period of increased activity and finally a return to the lag period.

When the maxima points of total growth are plotted, fig. 2 (diameter of colony against temperature), we have the relationship of the total growth to the various temperatures outlined.

These results seem to indicate that the optimum growth for this organism has changed from approximately 37.5° C. in its parasitic condition to 25° C. in a saprophytic condition. This latter fact will be demonstrated in the animal inoculation experiments. It also shows that the longer an organism is kept in culture the greater will be its change in physiological phenomena to an optimum point for its changed environment.

Hydrogen-Ion Concentration.—The problem of hydrogen-ion concentration has been of interest for several years and its

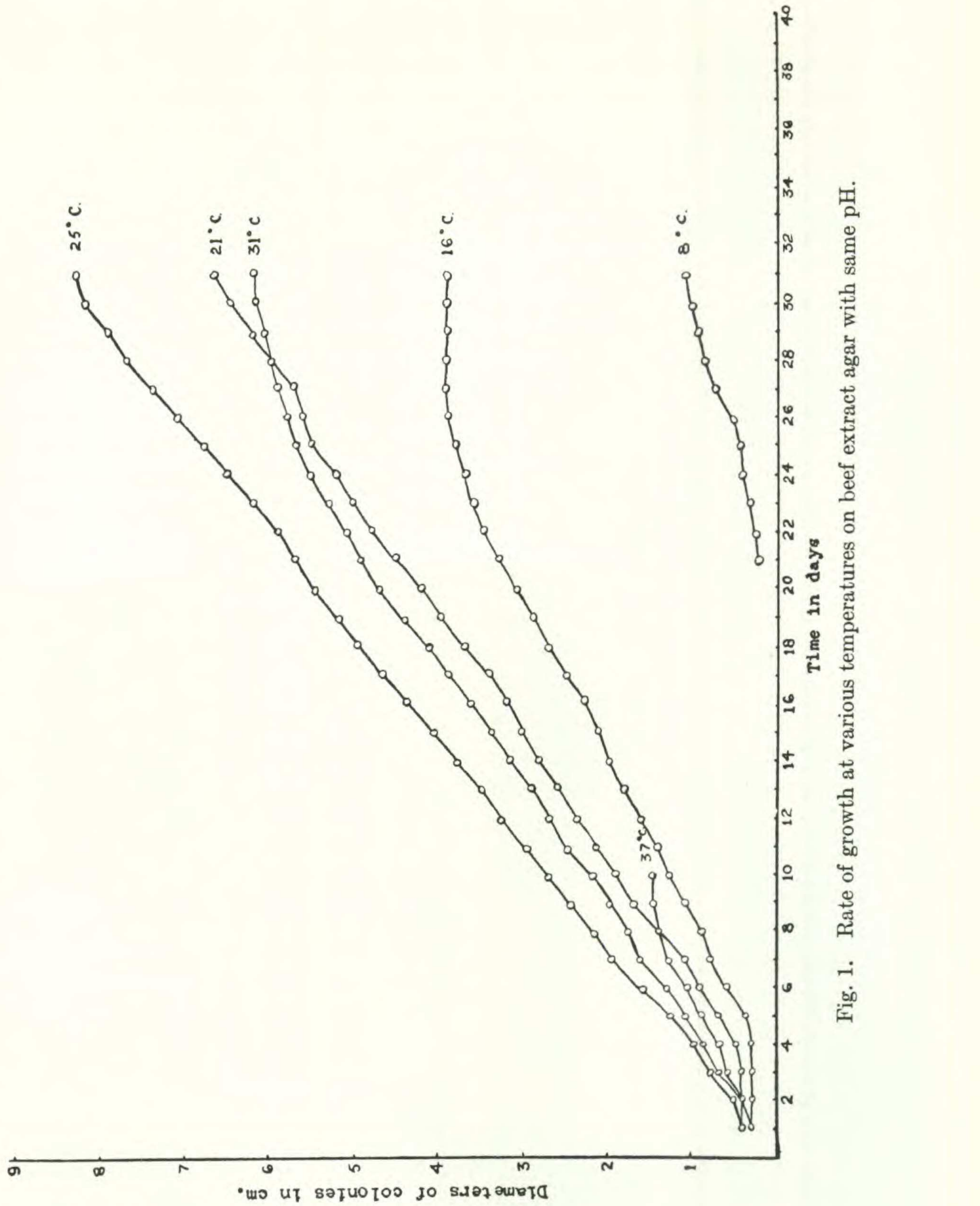


Fig. 1. Rate of growth at various temperatures on beef extract agar with same pH.

consequences especially emphasized by von Mallinckrodt-Haupt ('32). It has been demonstrated that the change of pH in a medium plays an important part in the cultural characteristics

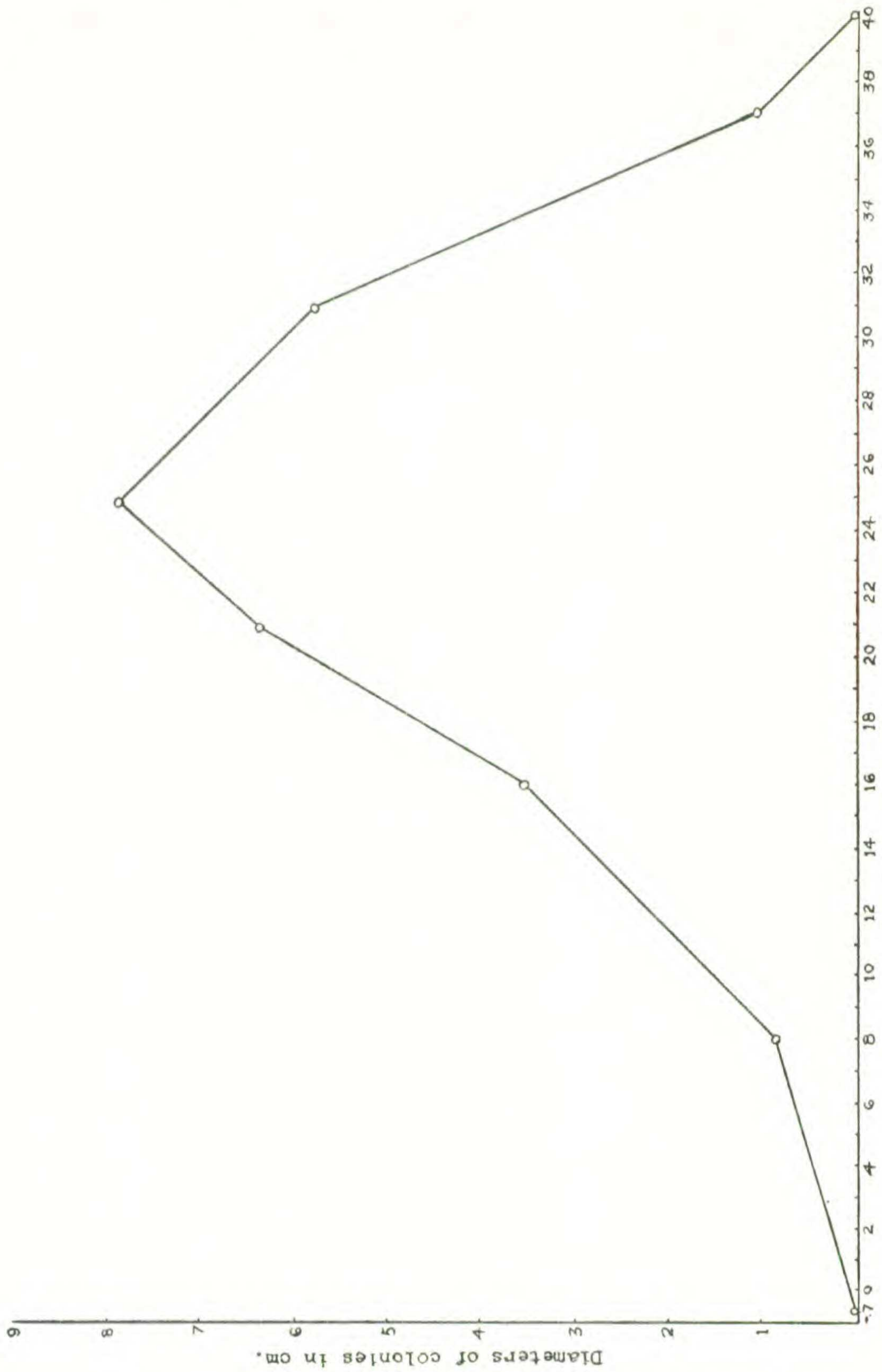


Fig. 2. Maximum growth at various temperatures on beef extract agar with same pH.

of the fungi grown. These changes may be brought about in their nutrient medium through the formation of acids which are

present in large amounts, by the breakdown of the sugar molecule in its utilization by the organism, by the decomposition of the proteins or the products, and by the cracking of fats or lipoids as a result of growth. As is perhaps fairly evident in the case of the carbohydrate reactions to be explained later, we have the formation of alkaline bases which are observed in the course of the utilization of the proteins.

From a study of the cultural characteristics mentioned previously and from further observations, a decided difference in growth on acid and alkaline media may be pointed out. In strongly acid media there is abundant budding and a formation of numerous conidia, and on strongly alkaline media a tendency towards shorter, thicker cells and a yeast-like cell formation. On the other hand, on weakly acid or alkaline media there is a favorable condition established for mycelial and hyphal formation.

The effect of alkalinity and acidity on the development of fungi was noticed for a number of years. Marantonio ('93) found that a more acid medium favored a greater quantity of mycelium. These observations were confirmed by Concetti ('00) and were extended to include a large number of media. Strains of an organism isolated from cases of thrush were used. Fineman ('21), working with *Monilia albicans* apparently, found that mycelium grew better in media under low surface tension and oxygen tension while the yeast form of the organism predominated on solid media, simple carbohydrates, and a high pH. Milochevitch ('29) found that varying the hydrogen-ion concentrations of media had no effect on the same sort of a fungus. Talice ('30) found the best development of hyphae on dilute potato decoction, the hydrogen-ion concentration being perhaps fairly high.

Several workers have pointed out that fungi possess a certain buffer action, that is, they have the ability to regulate their growth according to the reaction of the medium, and it is found to exist only under external changes. This means, then, that acid media become less acid during the course of growth and alkaline media become less alkaline.

What has been found to be true for the effect of various temperatures on the growth of *E. capsulatus* may also be said to hold with regard to hydrogen-ion concentration. For testing this,

beef extract agar made up with Liebig's extract of beef but adjusted to the various pH's shown in table II, was inoculated as described previously. The same procedure was carried out as in the above experiments, namely, daily diameter measurements and occasional microscopic examinations. Here, as before,

TABLE II

MEAN DIAMETERS IN CENTIMETERS OF COLONIES GROWN IN VARIOUS HYDROGEN-ION CONCENTRATIONS, AT A TEMPERATURE OF 26° C.

Beef extract agar (Liebig's extract of beef)

Days after inoculation	pH values												
	2.1	3.3	4.2	5.2	5.5	6.1	6.4	7.0	7.4	7.7	8.2	9.3	10.4
1	0.2	0.3	0.3	0.3	0.3	0.3	0.3	0.4	0.3	0.4	0.3	0.4	0.4
2	0*	0	0.3	0.3	0.3	0.3	0.3	0.4	0.3	0.4	0.3	0.4	0
3	0	0	0.3	0.4	0.4	0.4	0.4	0.6	0.5	0.6	0.4	0.4	0
4	0	0	0.3	0.5	0.5	0.6	0.7	0.9	0.8	0.9	0.6	0.5	0
5	0	0	0.3	0.7	0.8	0.9	0.9	1.2	1.2	1.2	0.8	0.7	0
6	0	0	0.3	1.0	1.1	1.2	1.2	1.4	1.6	1.6	1.1	0.9	0
7	0	0	0.3	1.3	1.3	1.5	1.5	1.7	2.0	1.9	1.4	1.1	0
8	0	0	0.3	1.4	1.6	1.8	1.9	2.1	2.3	2.2	1.8	1.3	0
9	0	0	0.3	1.7	1.9	2.1	2.2	2.3	2.7	2.5	2.0	1.5	0
10	0	0	0.4	2.0	2.2	2.4	2.4	2.6	3.0	2.8	2.4	1.8	0
11	0	0	0.5	2.3	2.5	2.7	2.7	2.9	3.3	3.2	2.7	2.0	0
12	0	0	0.6	2.6	2.8	3.0	3.1	3.2	3.7	3.5	2.9	2.2	0
13	0	0	0.8	2.9	3.1	3.3	3.4	3.6	4.0	3.8	3.2	2.3	0
14	0	0	1.0	3.1	3.4	3.5	3.7	3.8	4.3	4.1	3.5	2.4	0
15	0	0	1.1	3.3	3.6	3.8	4.0	4.1	4.6	4.4	3.8	2.4	0
16	0	0	1.3	3.7	3.9	4.2	4.2	4.5	4.9	4.7	4.1	0	0
17	0	0.4	1.6	4.0	4.3	4.5	4.6	4.8	5.3	5.0	4.4	0	0
18	0	0.6	1.8	4.3	4.5	4.8	5.0	5.2	5.6	5.4	4.7	0	0
19	0	0.8	2.1	4.6	4.8	5.1	5.3	5.5	5.9	5.7	5.1	0	0
20	0	0.9	2.3	4.9	5.1	5.4	5.6	5.9	6.3	6.0	5.4	0	0
21	0	0.9	2.5	5.1	5.3	5.7	6.0	6.2	6.6	6.4	5.6	0	0
22	0	0.9	2.7	5.3	5.5	6.0	6.3	6.5	6.9	6.8	5.9	0	0
23	0	0.9	2.8	5.4	5.6	6.2	6.5	6.8	7.2	7.1	6.2	0	0
24	0	1.0	3.0	5.7	5.9	6.4	6.7	7.0	7.4	7.2	6.4	0	0
25	0	1.0	3.1	5.8	6.1	6.6	6.9	7.2	7.6	7.4	6.5	0	0
26	0	0	3.2	5.8	6.3	6.8	7.0	7.4	7.8	7.6	6.7	0	0
27	0	0	0	5.9	6.4	6.9	7.1	7.5	8.0	7.8	6.9	0	0
28	0	0	0	6.1	6.5	7.0	7.2	7.6	8.2	8.0	7.0	0	0
29	0	0	0	6.2	6.6	7.1	7.3	7.7	8.3	8.1	7.1	0	0
30	0	0	0	6.3	6.7	7.1	7.4	7.7	8.4	8.2	7.1	0	0

*0 indicates no growth

contaminations were present, so that only three plate measurements were incorporated in the table. The mean results in table II represent a typical set of data in this work.

An analysis of the data shows that no growth took place at a pH of 2.1 and 10.4. At pH 3.3, growth was not evident until the seventeenth day, when the diameter was 0.4. The total growth was 0.7 cm. on the twenty-fourth day, after which it stopped. A transfer of the culture to a medium of pH 7.2 gave normal good growth. With an increase in pH to 4.2, growth was at first slow, but the colony attained a diameter of 3.2 cm. on the twenty-sixth day and then growth stopped, showing a total increase of 2.9 cm. In like fashion, by subtracting the initial inoculum measurement from the final colony diameter, we find that at pH 5.2 total growth for thirty days was 6.0 cm.; for pH 5.5, 6.4 cm.; for pH 6.1, 6.8 cm.; for pH 6.4, 7.1 cm.; for pH 7.0, 7.3 cm.; for pH 7.4, we find the optimum pH with a total growth of 8.1 cm.; for pH 7.7, 7.8 cm.; for pH 8.2, 6.8 cm., which is the same as that for pH 6.1; for pH 9.3, total growth of 2 cm. was acquired on the fifteenth day, with the colony showing no additional growth.

By plotting the diameter of the colony against the time in days, fig. 3, we obtain the rate of growth for the optimum pH which shows, except for the initial lag and a final lag, the latter being due perhaps to external factors as slight drying and probable utilization of most of the nutrients, an almost straight line. This varies with the pH, showing a decreased growth rate from the optimum, with an increased lag and decreased period of activity.

By plotting the maxima points of growth or total growth for each pH against the pH values, we obtain a clear relationship of the growth at various hydrogen-ion concentrations, with the point of optimum growth forming a peak, as seen in fig. 4.

In addition, the pH of several of the media after growth was determined. No definitely accurate results were obtainable due to the dried-up condition of several of the media, but indications seemed to be that there was an increased pH where growth was best, with no change in the strongly alkaline and strongly acid. The results may be interpreted by considering the amount of growth as an index of the production of alkali due to the utilization and decomposition of the protein substances in the medium.

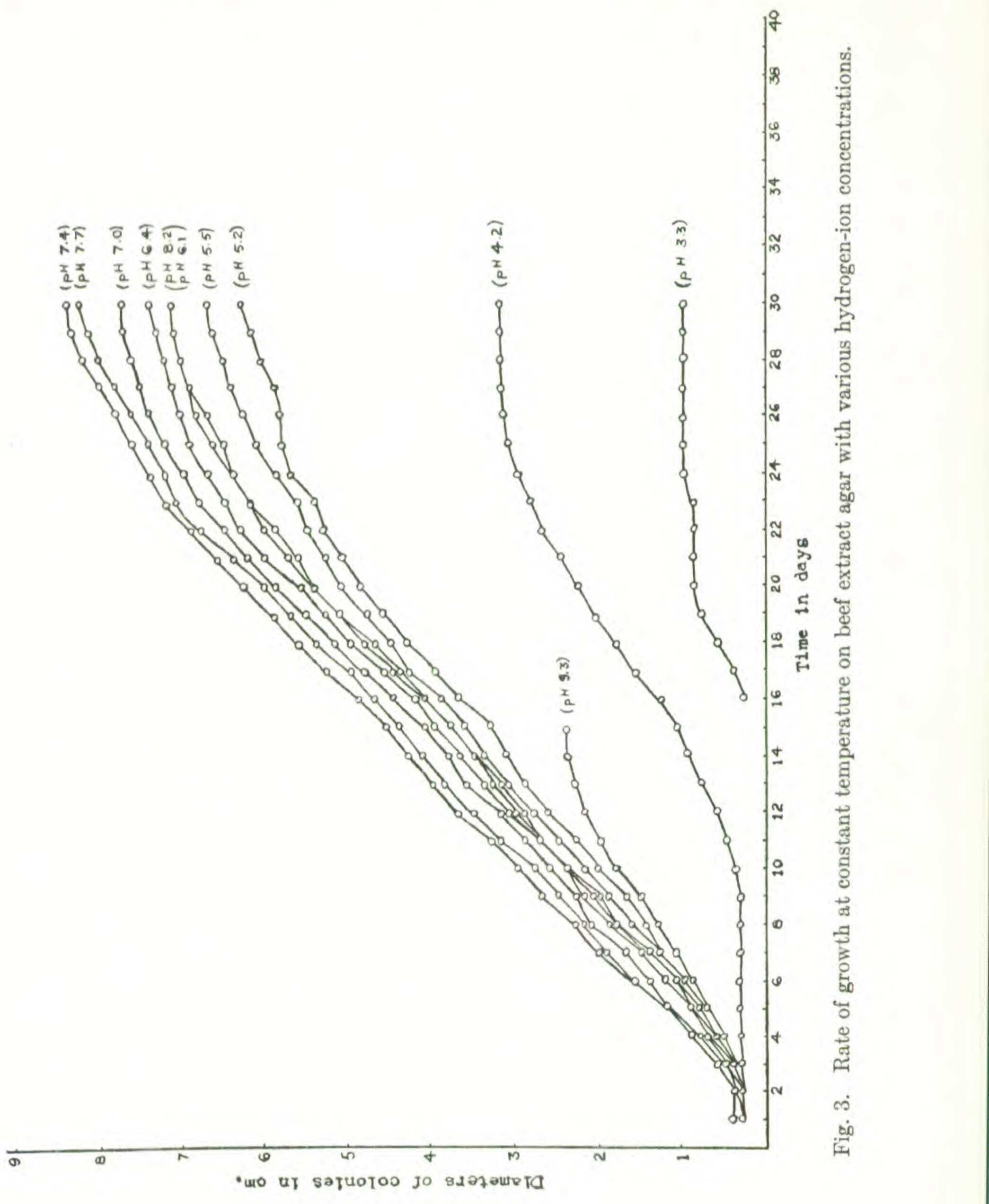


Fig. 3. Rate of growth at constant temperature on beef extract agar with various hydrogen-ion concentrations.

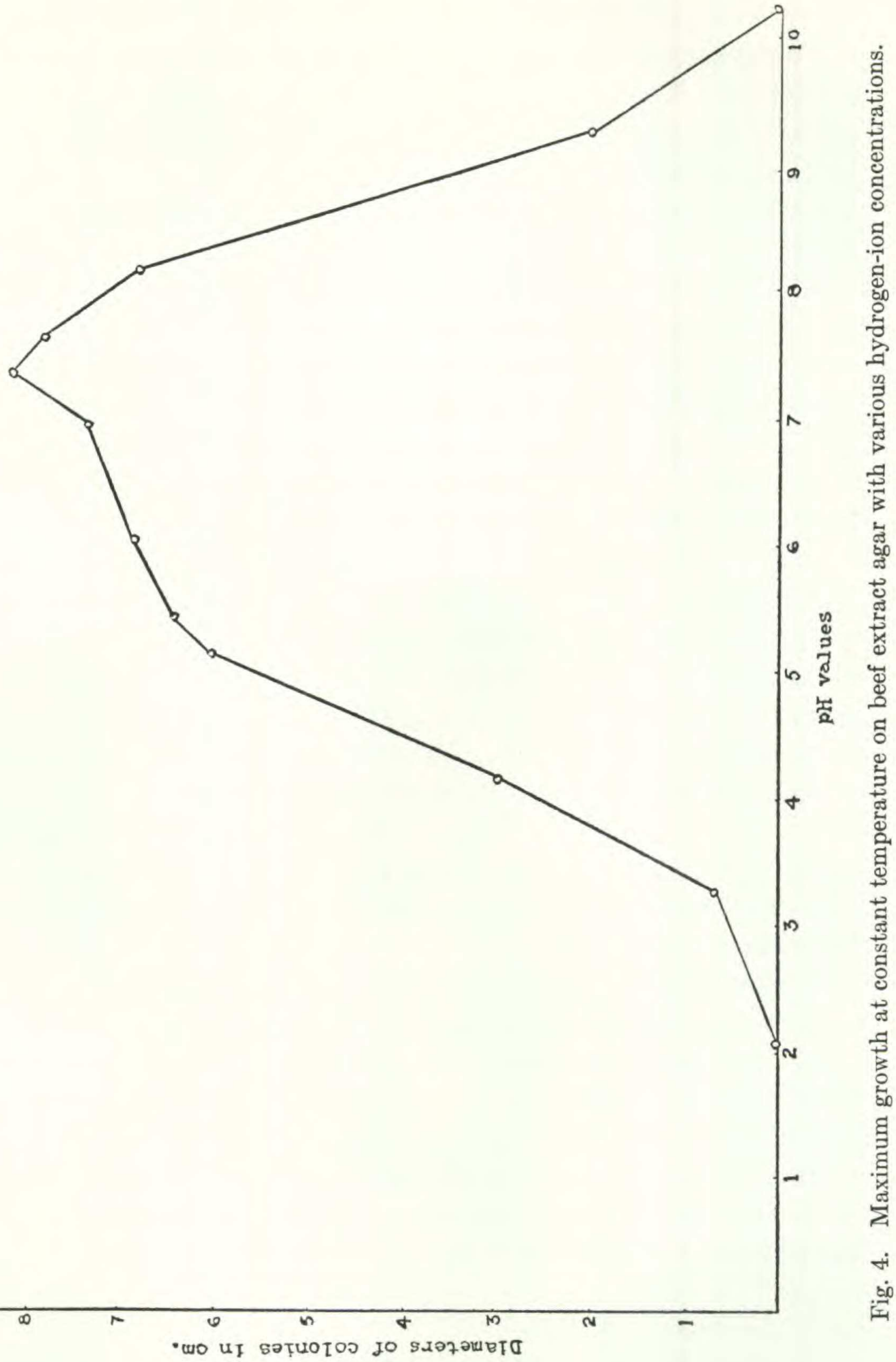


Fig. 4. Maximum growth at constant temperature on beef extract agar with various hydrogen-ion concentrations.

SUMMARY OF CULTURAL WORK

The results of the cultural studies described above for the three organisms, *Endomyces capsulatus*, *E. capsulatus* var. *isabellinus*, and *E. dermatitidis*, but more especially for *E. capsulatus*, may be summarized as follows:

1. Ability of the organisms to grow on a fairly wide variety of media.

2. No growth or very little growth on media lacking or having very little protein or carbohydrate.

3. An increase in the number of conidia and decrease in diameter of hyphae on acid media as compared with fewer conidia and increased thickness of hyphae on media with a high pH. The number of conidia and size of cells may be an index of the hydrogen-ion concentration of the medium.

4. *E. capsulatus* and its variety *isabellinus* show an almost direct change from a yeast-like to mycelium growth macroscopically, as evidenced by the change from a moist colony to a filamentous colony, whereas *E. dermatitidis* shows a macroscopically evident secondary stage in the form of a definite, prickly or coremium-like growth. The secondary stage is identical microscopically, for all the organisms.

5. *E. capsulatus* has a white color, *isabellinus* a light chamois or isabella, while *E. dermatitidis* has a dark brown or cinnamon color.

6. Size and number of various morphological characteristics differ on various media.

7. Reversion to the yeast-like form by growth on serum agar.

8. No growth on litmus milk.

9. The organism shows an optimum temperature of 25° C. with no growth occurring at temperatures above 37° C. and below 8.0° C.

10. The optimum pH is shown to be approximately 7.4 with deviations from the mean, and no growth occurring in a pH higher than 9.3 or lower than 3.3.

BIOCHEMICAL REACTIONS

Carbohydrate Reactions.—There have been many conflicting results in the past with regard to the carbohydrate reactions of yeasts and yeast-like organisms. Systems of classification have

been established whereby an organism was placed in a definite taxonomic position because of its production of either acid or gas, or both, or whether or not it had an effect on a carbohydrate. Such a system has been established by Castellani for *Monilia*. Whether any confidence can be placed in a method of that sort is doubtful, first, because it is problematical, at least in some cases, whether absolutely pure sugars are obtainable, and small amounts of impure substances may bring about an altered reaction which may not coincide with the specified phenomenon. That is, a reaction may be indicated as slight or weak, which actually should have been negative. Dekker in 1931 outlined the various methods of sugar fermentation and the faults to be found with each, and for further information, the reader is referred to her work. In the second place, it has been evidenced in the past and frequently noticed at present, that pathogenic organisms of the sort mentioned above have often lost their fermentative abilities on standing in artificial media. The constant subculturing has resulted, as in the case of many fungi, in a change from a pathogenic and virulent parasite to a non-harmful saprophyte, and while this may not necessarily be an index of the sugar reactions of an organism, still we must consider such occurrences as of some significance. It would seem, therefore, that not much reliance must be placed on a system of that sort.

In the work carried out here, stress was laid not so much on the desire to organize and establish a new method of classification, such being altogether too numerous, but to determine whether a yeast-like organism, such as *E. capsulatus*, could produce any acid or gas. For this purpose, Pfanstiehl sugars, which have been found to be considerably above the average, were used. The sugar was added, at the rate of one per cent, to a beef extract broth as prepared previously, to which had been added phenol red as an indicator. The phenol red was made up as a 0.02 per cent solution in distilled water and added in the amount of 1.5 cc. of indicator to 10 cc. of medium. This gave a fairly deep color which proved satisfactory in the work. This particular dye was chosen because preliminary experimentation had shown that the reaction tended more towards alkalinity. Litmus was also used in several series, but the results were not so clear and definite as with phenol red.

A 12-day-old culture on beef extract agar was mashed up with a sterile needle and 5 cc. beef extract broth of the same pH were added to form a suspension. A loopful of this supension was inoculated into Smith fermentation tubes which contained the above medium. All the tubes were grown at 25° C.

The carbohydrates used were those listed in table III. For the sake of clarity, they may be outlined as follows:

Monosaccharides.
- Pentoses:
  - Aldoses;
    - *l-arabinose, l-xylose*
- Hexoses:
  - Aldoses;
    - *rhamnose, dextrose, d-galactose, d-mannose*
  - Ketose;
    - *d-levulose*

Disaccharides.
- *lactose, maltose, saccharose*

Trisaccharides.
- *raffinose*

Colloidal polysaccharides.
- *dextrin, starch (soluble), inulin*

Glucosides.
- *amygdalin, salicin*

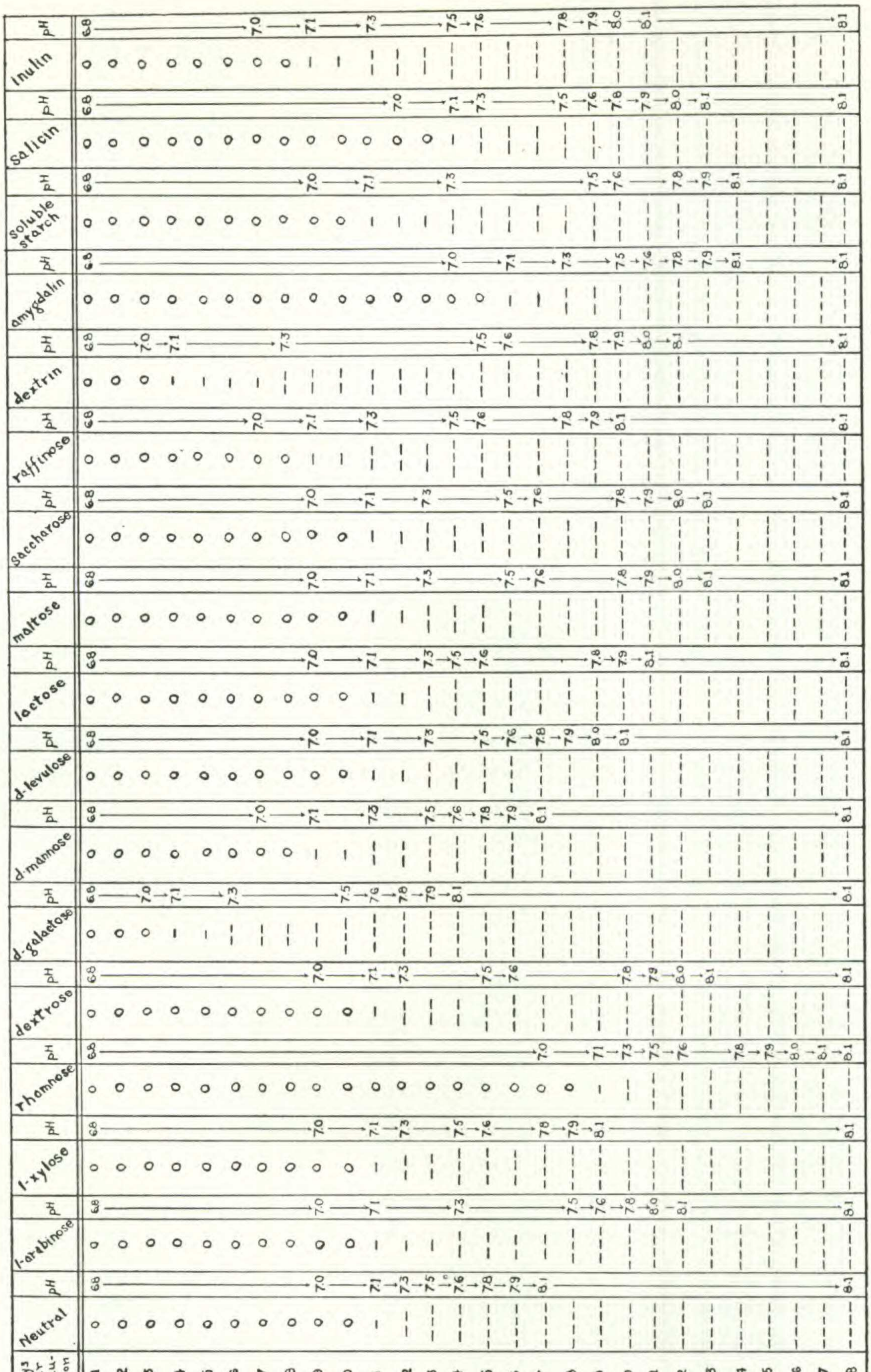
These carbohydrates represent a fairly wide range and include those sugars which are used in the regular routine sugar reactions, as xylose, dextrose, maltose, and lactose.

There will be no attempt at present to explain the work in detail or to offer any complex chemical formulae for possible decompositions, but the reactions and a possible empirical reason for the observations will simply be pointed out.

An examination of table III in which the carbohydrates are arranged in the order of their increasing groups, shows that when compared with the control (the same medium minus the carbohydrate) in the first column there was a changed reaction due to the presence of a carbohydrate. It will be noticed that the control tube showed a change on the eleventh day and a period of reactivity for six days, reaching its maximum on the seventeenth day after inoculation. It is further seen that 1-arabinose, 1-xylose, dextrose, d-levulose, lactose, maltose, saccharose, and soluble starch showed the same initial period of definitely de-

TABLE III

CARBOHYDRATE REACTIONS



monstrable reactivity, by the color changing to red, and that rhamnose showed a lag and hindered the reaction greatly before the color change, this being demonstrable on the nineteenth day. Amygdalin had a somewhat less harmful effect than rhamnose, with the reaction occurring on the sixteenth day, and salicin even less than that, having the initial color change on the fourteenth day. In contrast with the normal, we find that d-galactose and dextrin were very favorable towards color production, inducing a reaction on the fourth day, whereas d-mannose, raffinose, and inulin took effect in perceptible color changes on the ninth day.

It might be added here that three series of tubes were used in each experiment, two series being used to obtain the changes in pH, which were measured by a potentiometer and also a set of colorimetric standards. A set of tubes was prepared with phenol red at various hydrogen-ion concentrations. This method proved very satisfactory for further work, being much more rapid and fairly accurate since readings were made only to the first decimal place. It was found in practically every case that a change in hydrogen-ion concentration occurred two days before a color change, except in the case of d-galactose and dextrin where the reaction was more rapid, taking place on the third day. This latter reaction may be explained somewhat by the fact that the two carbohydrates are very favorable to growth, since the color change is due to the growth of the organism which brings about a reaction in the medium as a result of the utilization of the nutrients.

A striking feature of these reactions is the varied range of reactivity as may be seen in fig. 5. No explanation, outside of the rate of utilization of the carbohydrate, can be given at the present time for this phenomenon. As compared with the control, the range for dextrin is very large. By the range of reactivity is meant the period over which the color changes and the decrease in hydrogen-ion concentration take place, coming to an end point of an approximate pH of 8.1. The various carbohydrates present various ranges which are clearly evident in fig. 5.

Soluble starch, added to the solution at the rate of 0.2 per cent, showed a partial hydrolysis on the nineteenth day as evidenced

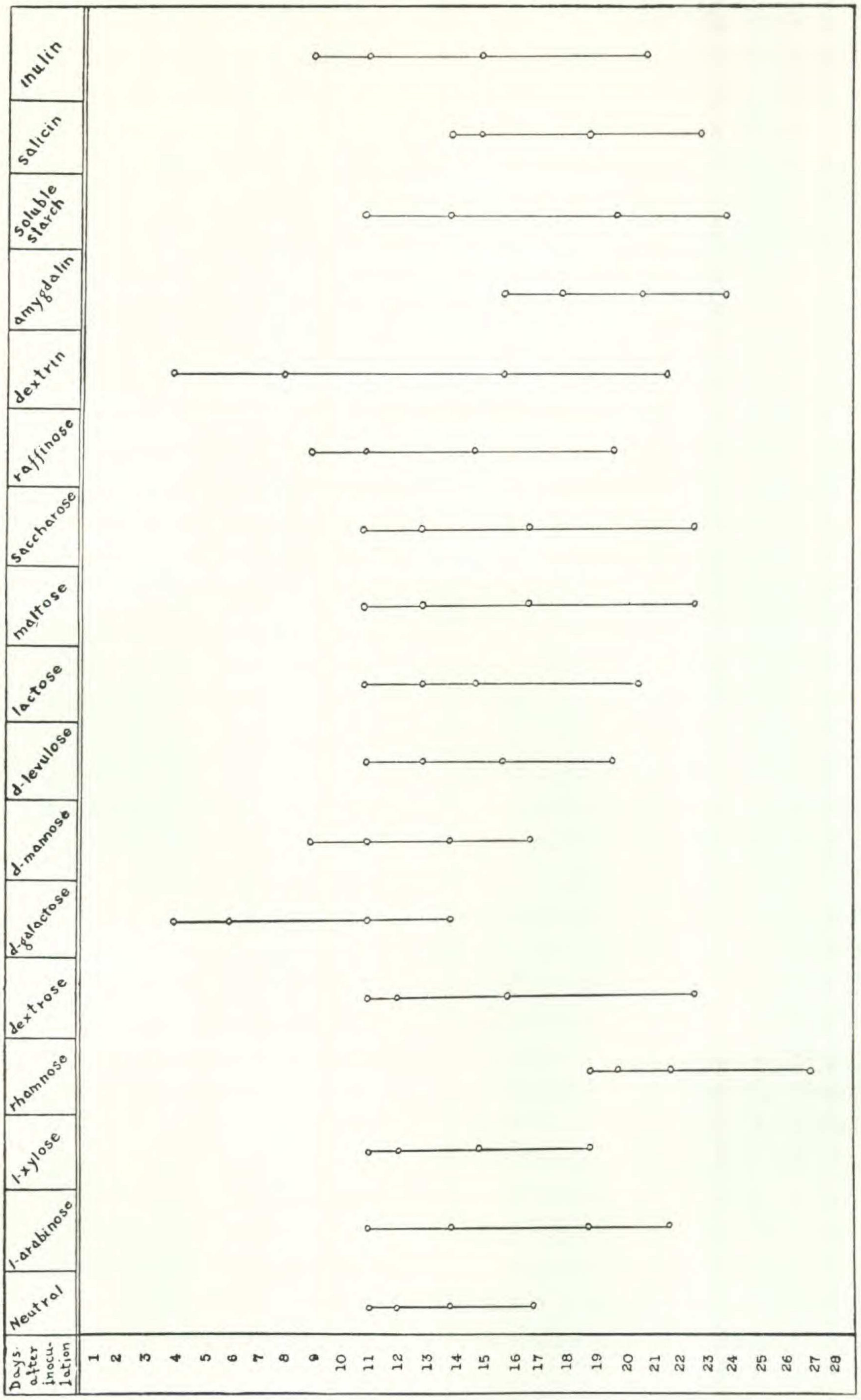


Fig. 5. Range of reactivity of carbohydrates.

by a reddish-brown color on addition of a dilute iodine solution. Fehling's solution reduced to cuprous oxide as shown by a faint reddish-violet color. There was no complete hydrolysis at the end of the experiment.

It may also be noted that in its production of alkalinity the reaction proceeded in every case from the aerobic portion of the Smith tube, or rather from the bulb, to the arm of the tube, or the closed portion. In other words, there was a diffusion of ions from the aerobic to the anaerobic. This of course was to be expected, since the organism is strictly aerobic, as will be shown later, and utilized all of the little material available, before growing towards the anaerobic portion. In every case, however, no matter how marked the reaction, the color change in the arm was comparatively slow.

Proteins are broken down very easily by reacting substances, and the cultural experiments have shown that *E. capsulatus* requires some protein, its derivatives, or carbohydrate, for growth. It is also known that these proteins and their derivatives may be further reduced or broken down to amino acids, peptones, peptides, and hexone bases and other substances, whereas higher carbohydrates may be reduced to simpler sugars. Ammonia is often found to be a product in the hydrolysis of proteins. In the presence of sugars, amino acids have characteristic reactions, particularly in an alkaline solution, and these have been dealt with by several authors, recently by Watanabe ('32). This author lists many reactions and covers the literature of the field rather well, and the reader is referred to his paper for detailed information. Euler and his associates, in some work carried out in 1926 and 1927, found that the reaction between amino acids and sugars which were reversible, took place at an equimolecular ratio and that their velocity became greater in an alkaline medium. This may account for the rate of activity as noticed in table III. The work of several others seems to point to the conclusion that a reaction between amino acid and sugar requires a neutral reaction and a fairly high temperature, whereas Waldschmitt-Leitz and Rauchalles observed that the optimum hydrogen-ion concentration for the reaction between dipeptides and glucose was pH 8.1. This seems to coincide with the results obtained here.

It is clearly evident from the experimental data that, instead of acid or gas or both, the carbohydrates here used induced the production of an alkaline condition. In addition to the protein decomposition substances as mentioned above, ammonia may be an end product. This substance, where present in fairly large amounts, brings about a strongly alkaline condition. To test for it, several cc. of the culture broth were poured into a test-tube, and a pinch of anhydrous sodium carbonate (Na_2CO_3) was added. The tube was shaken and a rather strong odor of ammonia was given off. It is possible that, besides the evident ammonia, hexone bases, as arginine, lysine, and histidine, which are alkaline in solution, are formed as products of the protein derivatives in the medium and that in addition to the sugar reaction they cause a decrease in the hydrogen-ion concentration.

Indol and Skatol.—Reactions which have long been used in bacteriological technique but which have not received much attention until lately, due to the additional knowledge and better methods which are applied, are those of indol and skatol. The work of such investigators as Pittaluga ('08), Goré ('21), Holman and Gonzales ('23), Morelli ('09), Sasaki ('10), Zipfel ('12), Krumwiede and Pratt ('13), Fellers and Clough ('25), Koser and Galt ('26), and several others has greatly added to the methods of technique in investigations. Tests have been advocated from time to time, the importance of which lay in their specificity for the reaction. Fellers and Clough list about thirteen tests which have been applied, some successful some not.

Indol and skatol are protein derivatives and their determination rests on the presence of tryptophane. From it they are broken off by a deamidization to form first indol proprionic acid, then indol acetic acid, and finally indol or skatol. The medium to be used in such a reaction must therefore contain a supply of tryptophane sufficient to determine whether the organism would actually be capable of breaking it down to indol or skatol or both. For this purpose, it was necessary to use peptone plus 0.1 per cent casein in water to insure good growth and also to comply with the requirements as stated. The broth was inoculated and the organism was allowed to grow at 25°C.

In the first test the Gnezda ('99) oxalic acid principle was

applied. Strips of filter paper were placed in a warm solution (saturated) of oxalic acid. On cooling, crystals of oxalic acid remained on the strips. The paper was then dried well and inserted in the mouth of the flask under aseptic conditions, so that it was pressed against the side of the flask and just above the surface of the broth. If indol is formed it volatilizes and colors the oxalic acid pink. In these tests for *E. capsulatus*, a very faint pink was barely discernible. This test has been advocated rather strongly by the Society of American Bacteriologists for regular routine work in bacteriology.

A test that was applied successfully is the Ehrlich-Böhme technique which consists of two solutions. Solution 1 contains 1 gm. para-dimethyl-amino-benzaldehyde, 95 cc. ethyl alcohol (95 per cent), and 20 cc. hydrochloric acid, concentrated. Solution 2 is a saturated solution of potassium persulphate ($K_2S_2O_8$). In the experiment, 5 cc. of solution 1 are added to 10 cc. of the culture fluid, then 5 cc. of solution 2, and the mixture is shaken. A faint red color appearing about five minutes later indicated a weakly positive reaction. This method has proven rather successful for indol. Steensma ('06, '06a) substituted vanillin for the para-dimethyl-amino-benzaldehyde, but this was not applied here.

The Salkowski ('83) nitroso-indol test was negative for indol.

Skatol was tested for by the Sasaki ('10) methyl alcohol test. This method consisted of adding to the broth to be tested about four drops methyl alcohol and an amount of concentrated sulphuric acid equal to the broth. The acid contained a trace of ferric salt. A reddish-violet color indicated a positive skatol reaction.

Under the proper conditions, indol and skatol may be produced by *E. capsulatus*.

Relation to Free Oxygen.—Aerobiosis has often been considered important in experimental work. Organisms are classified as strict aerobes, facultative aerobes, or facultative anaerobes and strict anaerobes. In order to find the position of *E. capsulatus* in this classification, tubes of broth were prepared and inoculated by the Liborius method (Zinsser, '29) and kept at 25° C. Cultures were kept at the same temperature and in the

same broth, but were plugged only, allowing oxygen to enter. No growth occurred in the former while a luxuriant growth occurred in the latter. Agar stab cultures were negative for growth in the stab, but positive on the surface. The relationship to free oxygen was also demonstrated in the carbohydrate reactions as mentioned previously.

We may conclude, therefore, that *E. capsulatus* is a strict aerobe, at least in so far as its growth on agar after a period of time has demonstrated. When in the yeast form, anaerobic cultures showed slight growth, a condition of facultative anaerobiosis. Continued growth on an artificial substrate has converted it into a strict aerobe.

Reduction of Nitrates.—The tests for nitrate reduction as advocated by the American Society of Bacteriologists were inconclusive and unsatisfactory. Ammonia was found to be present, but that was apparently due to the breakdown of the peptone and other protein decomposition products. Trommsdorf's method was also indefinite, being negative in general, but at times gave a slightly colored reaction.

Production of Hydrogen Sulphide.—The formation of hydrogen sulphide in culture has often been concerned with a toxic action of the lead salt or lead acetate in the medium toward certain bacteria. Whether hydrogen sulphide would be produced here was of course problematical. However, a lead acetate agar put up by the Digestive Ferments Co., "Bacto lead acetate agar," which is claimed to contain too little lead salt to be toxic and yet was favorable for the demonstration of the hydrogen sulphide, was used. Making stab cultures was of course illogical, for reasons pointed out before, and so surface inoculations were made and the cultures incubated at 25° C. A slight brownish coloration was found to occur directly around the colony after thirty to forty days, and increased somewhat on standing.

No definite method has been advocated for the above experiment, and since the results were slightly evident even though the period was a great deal longer than for bacteria, the reaction might be considered positive.

Gelatine Liquefaction.—Tubes of 15 per cent meat extract gelatine were inoculated by stabbing and incubated at 25° C. A

slow liquefaction took place between 30 and 40 days, and in some cases even longer than that. Liquefaction began at the surface and proceeded downward. This may be correlated with the growth of the fungus.

REACTION TO LIGHT

A great deal of experimental work has been done in the past, and much is being attempted at the present, with respect to the

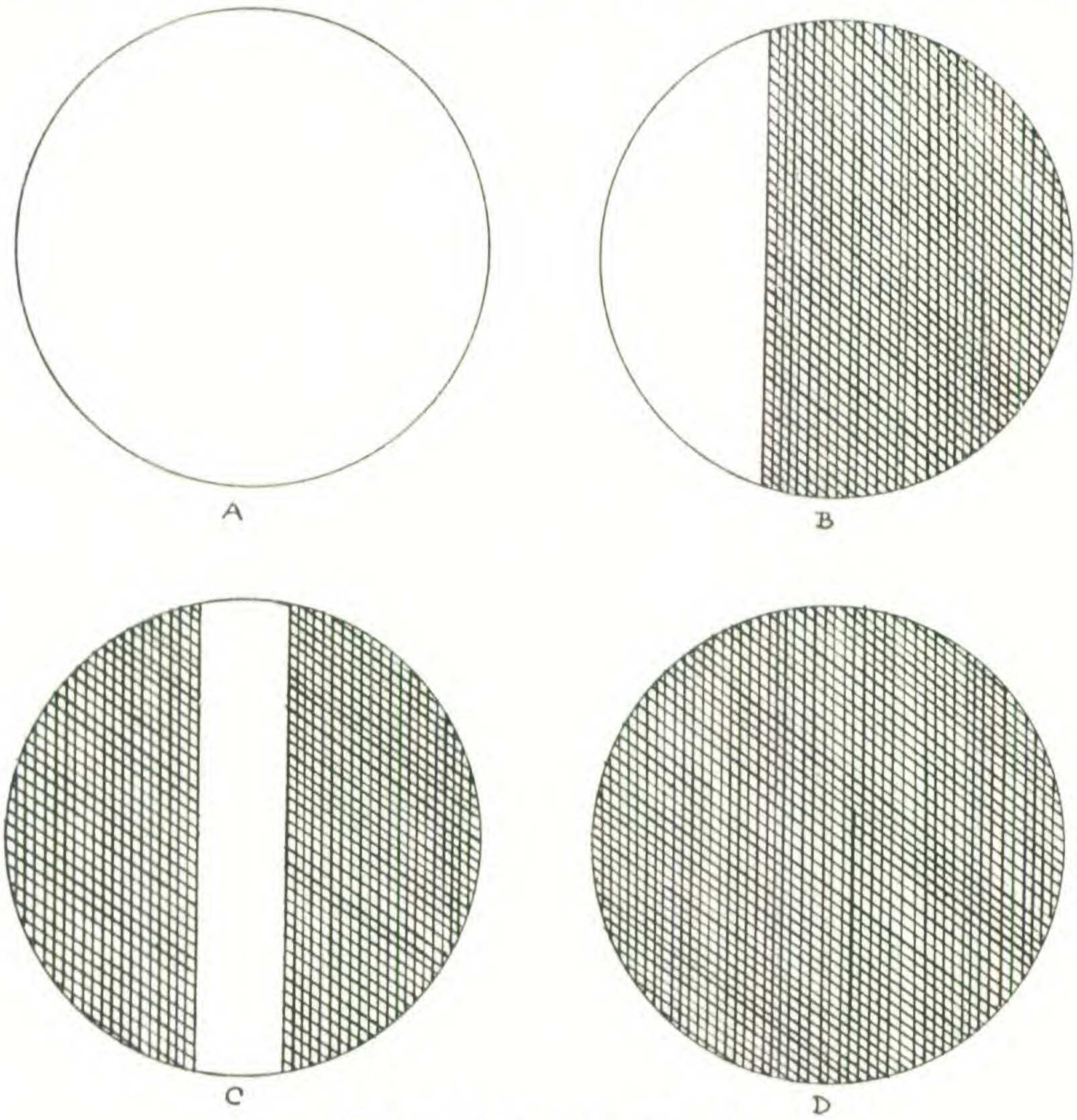


Fig. 6. Explanation in text.

action of light on higher plants and also microbiology. It is accepted as a fact by many that plant growth and vitality can be greatly altered, either for better or worse, by continued treatment under variously colored lights. No great amount of work, however, has been done on the action of the primary colors on fungi or bacteria. Several workers have found a destructive

action of light on the lower organisms, and Rahn has found that red and green light have no effect while blue light has a very harmful action, with the degree of destruction decreasing in the violet and ultra-violet portion of the spectrum.

Effect of White Light on Growth.—To determine the differential action of light and darkness on *E. capsulatus*, series of plates were

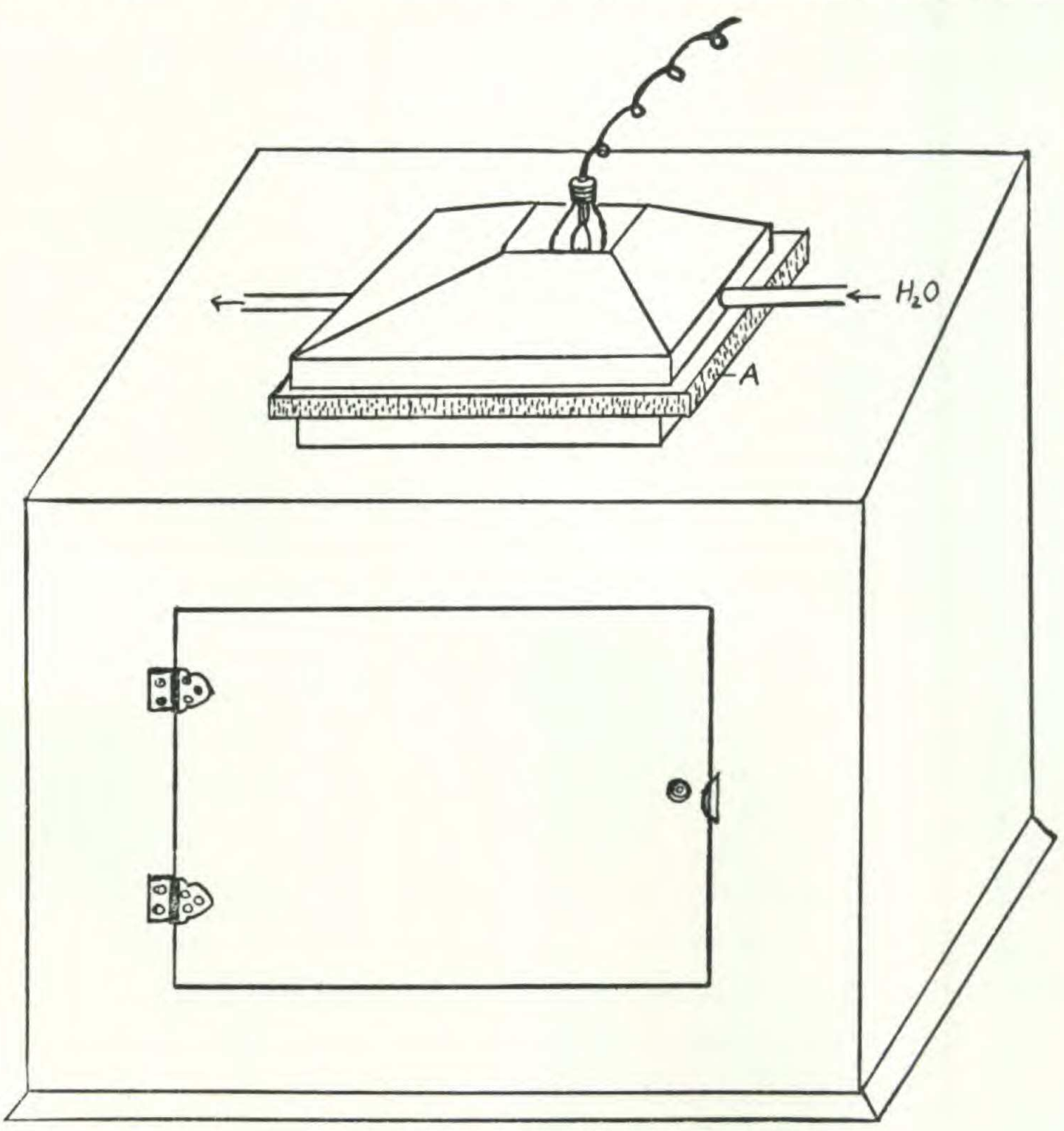


Fig. 7. Explanation in text.

covered with opaque black paper of the sort used in covering photographic plates, as illustrated in fig. 6, the portions with the crossing lines representing the covered areas. The plates were treated as follows: plate A was inoculated in the center and completely exposed to the light; plate B was covered as shown and was inoculated at a point about 1.5 cm. from the rim of the plate, or about 5 cm. from the uncovered area. This plate was used to determine whether the organism would grow towards the

light or not. The plates covered as in C were inoculated in the center of the portions covered, and plate D was inoculated as in plate A and served as the complete darkness plate. Nutrient agar was used. These plates were placed in a light-proof container illustrated in fig. 7, at room temperature. A General Electric mazda bulb of 50 watts and 115 volts was used as a source of light. A flow of water was kept constant beneath the source of light to prevent the heat from reaching the interior of the container. A sheet of colorless glass (A) was kept between the flowing water and the cultures. Several cultures of each series were kept in the container and the experiment was performed on several different occasions. Plates prepared as above were kept at room temperature and were subject to diffuse daylight and darkness. Growth at the end of 24 days showed no cultural or microscopical differences in any case, except for a slight increase in diameter of three plates kept in the freely circulating air. This small number of attempts seemed to indicate that light had no effect on growth.

Effect of Red Light on Growth.—It has been pointed out that red light stimulated growth in higher plants but, according to Rahn, had no effect on lower organisms. Shrewsbury found that when cultures of *Willia* were placed at a distance of 4 inches from a Wratten safelight No. 1 screen, illuminated by a 60-watt gas-filled lamp, with the temperature of the dark room ranging from 23 to 28° C., growth was more rapid and more luxuriant than in the dark or diffuse daylight, as expressed by a hastening of spore formation. He attributed this increased activity to a reflex of increased growth energy, with red light acting as a stimulant. However, he adds that much the same growth was obtained in cultures grown at 30° C. as in those grown at room temperature, so that it was very likely that the heat and not the red light was the activating agent.

To avoid this condition, the light-proof container as described above (fig. 7) was again used. This time a red bulb of the same intensity as before was used and a plate of red glass replaced the colorless glass. The same medium of the same pH was used here too. Cultures were grown at room temperature in the dark and diffuse daylight as controls. After 27 days it was found that

those cultures under red light displayed no cultural or microscopic differences from those grown in the dark and daylight, and showed no change from those in white light and total darkness, except for a small increase in diameter which may be ascribed to the extra time of 3 days.

Reaction to Dyes

The rôle of certain dyes in medical work has received much critical and experimental attention for a number of years. Their application for therapeutic purposes has resulted in clinical cures in various cases and has had no effect in others. Particular dyes, as methylene blue, gentian violet, and crystal violet, have been used locally or intravenously for skin lesions in cases of blastomycosis, coccidioidal granuloma, sporotrichosis, monilial infections, and other diseases both of mycological and bacteriological etiology. Some of these have yielded favorable results as mentioned, but the value of the dyes applied is empirical since dye therapy rests on no definite scientific basis, according to Spring ('29). Faber and Dickey ('25) treated 15 infants afflicted with thrush, with local applications of a 1 per cent aqueous solution of gentian violet and found that 50 per cent showed an apparent cure in one day or less; 36 per cent in from 2 to 3 days; and 14 per cent in from 4 to 5 days. In several cases, however, the lesions returned at various intervals, and again some disappeared after one application while others resisted for several applications. In general, the lesions seemed to become smaller after continuous applications and to disappear. As a result, Faber and Dickey advocate a trial of the dye for prophylactic measures and also for therapy. Churchman ('20d), working on chemotherapy with gentian violet *in vitro*, advised careful considerations of the amount of bacteria affected, before any conclusion as to the therapeutic value of the dye be evaluated. Sanderson and Smith ('27) postulated the possibility of the utilization of gentian violet dye for the treatment of blastomycosis.

It has been known and recorded since the days of Koch that certain aniline dyes have a "bacteriostatic" effect on the culturability of various bacteria. The viability of the organisms is

diminished to such an extent that they lose their power to multiply. This selective action of the dyes is very remarkable, certain organisms growing very abundantly and luxuriously on a medium containing gentian violet in a rather high concentration, whereas other organisms which are very resistant to harmful factors do not grow at all. This high selectivity or specificity was attributed not only to gentian violet, but also to others, as crystal violet and various members of the tri-phenyl methane series of dyes.

In early work on dyes, smears and broth cultures were used, but it was found that the bacteria ceased growing or were killed in the smears and that various concentrations of dye in the broth had practically the same effect. Later work (Churchman, '12) was performed on the divided agar plate to determine whether the reaction would be the same. This method consists of inserting a metal strip (Halsted's aneurysm metal) across the diameter of the petri dish and then pouring the agar on one side and allowing it to cool. The agar containing the dye is poured on the other side of the strip. When the agar hardens, the metal strip is lifted out with sterile forceps. Thus one half of the plate contains plain agar and the other half the dye-containing agar. This method has been used by several investigators, including Sanderson and Smith.

Churchman ('12,' 20, '20a, '20b, '20c, '20d, '20e, '21) found that a parallelism with the reaction of the Gram stain could be made. That is, considering as violet-positive those organisms which were inhibited in growth by a certain concentration of a dye, gentian violet for instance, and as violet negative those upon whose growth the dye had no effect, then it was found that a great number of Gram negative organisms were also violet negative. This is a general rule, with a few outstanding organisms being exceptions.

The same author also found that the selective action of the dye may be expressed in one of two ways. The dye may be toxic to the violet-positive organisms when a direct application is made, or a very strong inhibitory action is expressed when it is incorporated in the medium. On the other hand, the dye is not toxic to violet-negative organisms, and further, no inhibitory

action is expressed except in very strong concentrations when it is incorporated in the medium. His experimental evidence pointed to a dilution of 1 : 1,000,000 as capable of stopping growth, and 1 : 2,000,000 as possibly retarding it.

This work was confirmed by Krumwiede and Pratt ('13), who used dahlia agar, and also they confirmed the belief that the action was quantitative. They further observed that several dyes, in addition to those already mentioned, had this specific action. This work then called forth several applications of this principle to culture media, as that of Petroff ('15) which was used by that author to isolate tubercle bacilli from the sputum and feces, and that of Farley ('20) as a restrainer in the isolation of pathogenic molds. Farley found that a dilution of 1:500,000 inhibited the growth of Gram positive bacteria.

Later work on this problem showed that there might exist two types of organisms within a single strain, one which might grow vigorously on gentian violet media, and the other not at all, yet both showing the same stain and cultural characteristics. Also, heavy and repeated inoculations of violet-positive organisms would give fair and even good growth, and on this point Churchman ('20e) made the following statement: "This would indicate that bacteria do not, as is commonly supposed, act as isolated individuals; they possess the power, in numbers, of accomplishing effects which, alone, they are incapable of. The nature of this community of action it is at present impossible to guess at." The same author (Churchman, '21) placed dead bacterial bodies between living bacteria and gentian violet media and found that the Gram positive organisms grew, the dye having no effect. He attributed this occurrence either to a filtration or a stimulation of growth. The same year Churchman and Kahn ('21) found that a number of cells could accomplish more than a single cell, hence the communal action belief was further emphasized.

Work on dyes was not confined to bacteria alone, for Lewis ('30) found that a certain number of dyes failed to inactivate the virus of chicken tumor while many had a harmful effect in certain concentrations. Several papers have been published showing the action of certain of these dyes on various fungi, but only in a qualitative way; Greenbaum and Klauder ('22) tested the action of gentian violet

as advocated by Farley ('20a); Sanderson and Smith ('27) found that dilutions up to 1 : 500,000 inhibited *E. dermatitidis* in its yeast-like form; Clark ('27) showed that no growth occurred in a yeast-like organism and its mycelial form in dilutions from 1 : 100 to 1 : 25,000 and that it gradually increased from 1 : 50,000 to 1 : 1,000,000, which gave good growth. No quantitative determinations of the colony growth were made. Stearn and Stearn ('29) found that several fungi, including *E. dermatitidis*, were inhibited at various concentrations of gentian violet, with the organism being affected at 1 : 500,000.

The later work of Churchman indicated that the "bacteriostatic properties" of dyes ascribe to them the ability to interfere with the reproductive mechanism, which he terms "genesistasis," without killing or in any way interfering with their other properties. This same phenomenon has been found both qualitatively and quantitatively to hold for *E. capsulatus*.

Since the author could find that no particularly quantitative results had been obtained for ascomycetous fungi, at least for the hyphal form, it was decided to investigate the problem of the dye reaction. For this purpose, several dyes, picked at random, were used. These are as follows:

Dyes of the nitro, azo, and oxyquinone groups.
  Dyes of the azo group:
    orange G—acid—(National Aniline and Chemical Co.)
    Sudan III—weakly acid—(E. Merck)
  Dyes of the oxyquinone group:
    alizarin—acid—(E. Merck)
The quinone-imide dyes.
  The thiazins:
    methylene blue—basic—(Coleman and Bell Co.)
  The azins:
    Amido-azins or eurhodins;
      neutral red—weakly basic—(Coleman and Bell Co.)
    Safranines;
      safranine A—basic—(Coleman and Bell Co.)
The phenyl-methane dyes.
  Tri-phenyl methane derivatives:
    Di-amino tri-phenyl methanes;
      light green S. F. yellowish—acid—(Coleman and Bell Co.)
Tri-amino tri-phenyl methanes (rosanilins);
    basic fuchsin—basic—(Coleman and Bell Co.)
    crystal violet—basic—(Coleman and Bell Co.)
    aniline blue—acid—(National Aniline and Chemical Co.)

The xanthene dyes.
  Fluorane derivatives:
    eosine B—acid—(Coleman and Bell Co.)
  Phenolphthalein and the sulphonthaleins:
    phenol red or *phenol-sulphonphthalein*—acid—(Dr. T. Schuchardt, G. m. b. H. Chemische Fabrik)
The natural dyes
  Brazilin and haematoxylin:
    haematoxylin (Coleman and Bell Co.)

These dyes were incorporated in the media, nutrient agar (product of Digestive Ferments Co.) at a pH of 6.8, in the following concentrations: .00001; .000025; .00005; .000075; .0001; .00025; .0005. Several plates were used for each concentration of the dye, but only three plates for each are recorded in the tables for reasons stated previously. These plates were inoculated in the same manner as were those in the temperature and pH experiments, a broth of pH 6.8 being used here. The plates were kept at room temperature, which showed slight changes from day to day but not enough to affect the results seriously. The diameters of the colonies were measured daily at approximately the same hour each day. Inasmuch as the colonies may show slight irregularities in peripheral growth, these measurements may not be considered absolutely correct. However, if the shape of the colony was irregular, several diameters were measured and the mean taken as representative of that colony. Where contaminations occurred, as has been explained previously, the plates were discarded.

It is to be noted that these experiments were carried out on several different occasions and that the figures in the tables denote the mean of a representative series. Since preliminary experiments with these dyes had given some seemingly strange results, microscopic examinations were made daily, in the case of the crystal violet and methylene blue series, and then, some time after growth had started, in the case of eosine B.

Several of the dyes produced no effect on the growth of the organism. These dyes were orange G, Sudan III, alizarin (table IV), safranine A, light green, basic fuchsin, aniline blue, phenol red, and haematoxylin. Neutral red was also used, but, as was expected, the results showed no difference from the normal control, the same being true for the other dyes.

If normal conditions are maintained for the plates, that is, if there is no drying out and no change in temperature the colonies grow at a rate of speed which is fairly constant as long as there is sufficient nutrient material for the organism. However, when the plates are kept in an atmosphere which allows drying out of the agar and when the amount of nutrient material is relatively small for the particular organism, the colony will show a decrease

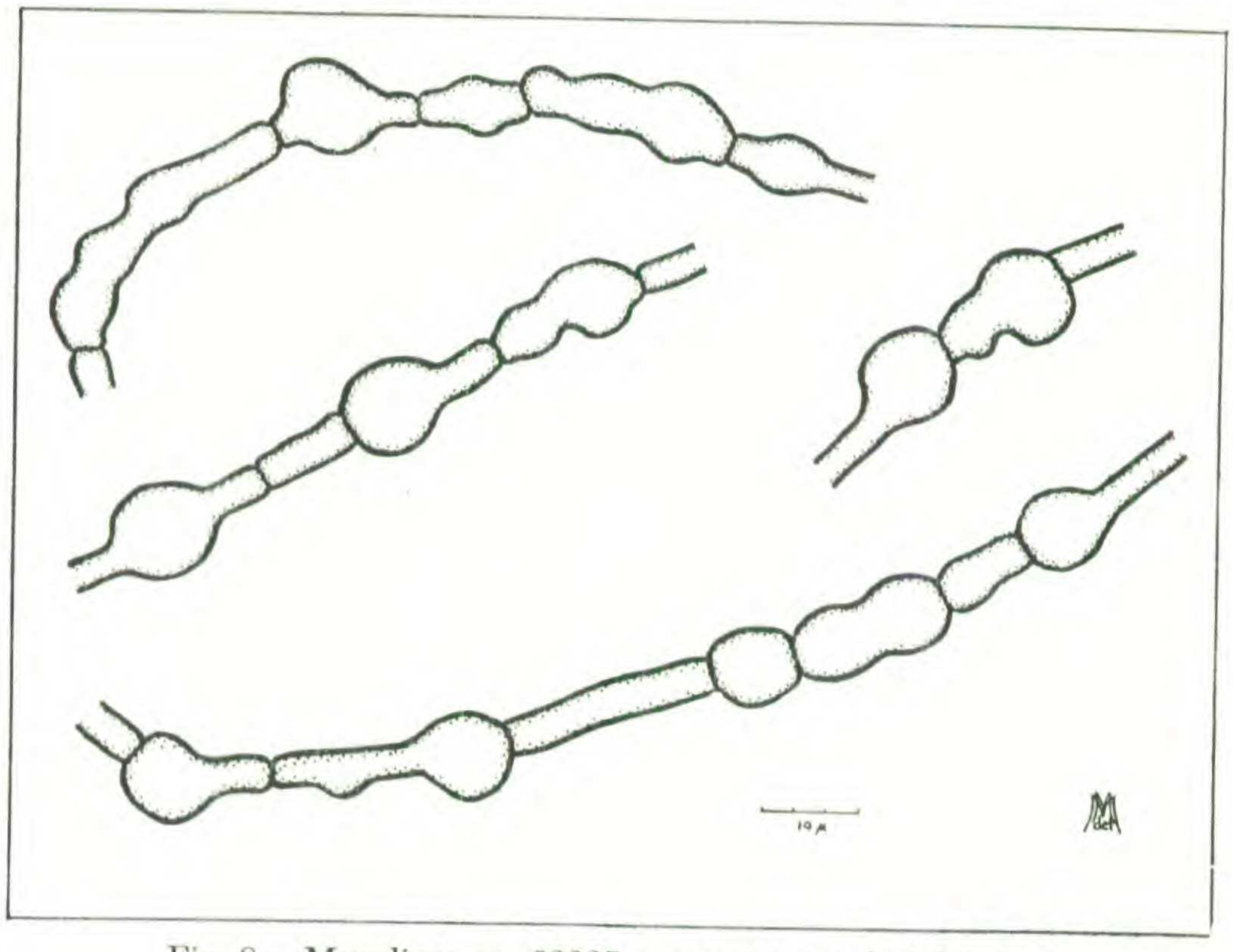


Fig. 8. Mycelium on .00025 per cent crystal violet agar.

in growth rate. Thus, in the former case, the growth curve would be a straight line for a considerable portion, depending of course on the rapidity of growth of the organism compared from day to day, which in this case was sufficient to cover the plate almost completely before drying of the substrate. An initial lag on artificial substrates, as agar, must be taken into consideration. In that case the curve would tend to be sigmoid, that is, there would be an initial lag due to an acclimatization of the organism to the medium, a portion of increased activity which would be a sharp rise, and then a portion of decreased activity due to factors mentioned previously.

The data of the growth on the several non-affecting dyes

TABLE IV

MEAN DIAMETERS IN CENTIMETERS OF COLONIES GROWN IN VARIOUS CONCENTRATIONS OF ALIZARIN, AT A TEMPERATURE OF APPROXIMATELY 22° C.

Nutrient agar (product of Digestive Ferments Co.) pH 6.8

Days after inoculation	Percentage concentration of dye						
	.00001	.000025	.00005	.000075	.0001	.00025	.0005
1	0.3	0.3	0.3	0.5	0.4	0.4	0.4
2	0.3	0.3	0.3	0.5	0.4	0.4	0.4
3	0.3	0.3	0.3	0.5	0.4	0.4	0.4
4	0.5	0.6	0.6	0.8	0.7	0.6	0.6
5	0.6	0.7	0.8	0.9	0.9	0.8	0.8
6	0.8	0.8	1.0	1.0	1.1	0.9	1.0
7	1.0	1.0	1.1	1.2	1.2	1.0	1.2
8	1.3	1.3	1.4	1.4	1.5	1.3	1.5
9	1.5	1.6	1.6	1.6	1.6	1.6	1.6
10	1.7	1.7	1.8	1.8	1.9	1.8	1.9
11	1.9	1.9	2.0	2.0	2.1	2.0	2.0
12	2.0	2.1	2.2	2.2	2.3	2.2	2.3
13	2.2	2.3	2.4	2.4	2.5	2.4	2.5
14	2.4	2.5	2.6	2.6	2.7	2.7	2.7
15	2.7	2.8	2.8	2.9	3.0	2.9	3.0
16	2.9	3.0	3.1	3.0	3.2	3.2	3.2
17	3.1	3.2	3.3	3.3	3.4	3.4	3.4
18	3.3	3.4	3.5	3.5	3.6	3.5	3.6
19	3.6	3.6	3.7	3.7	3.7	3.7	3.7
20	3.7	3.7	3.9	3.8	3.9	3.9	3.9
21	3.9	3.9	4.0	4.0	4.1	4.0	4.1
22	4.1	4.1	4.2	4.2	4.3	4.2	4.3
23	4.2	4.3	4.3	4.3	4.4	4.4	4.4
24	4.5	4.5	4.5	4.5	4.7	4.6	4.6
25	4.6	4.6	4.7	4.7	4.8	4.7	4.7
26	4.8	4.8	4.8	4.8	5.0	4.9	4.9
27	4.9	4.9	5.0	5.0	5.1	5.0	5.1
28	5.1	5.1	5.1	5.1	5.2	5.2	5.2
29	5.2	5.2	5.3	5.3	5.3	5.3	5.4
30	5.4	5.4	5.4	5.4	5.6	5.5	5.6
31	5.6	5.5	5.7	5.6	5.7	5.6	5.7
32	5.8	5.7	5.8	5.8	5.9	5.8	5.9
33	5.9	5.9	6.0	5.9	6.1	5.9	6.1
34	6.1	6.1	6.2	6.1	6.3	6.1	6.2
35	6.2	6.2	6.3	6.2	6.4	6.2	6.4
36	6.4	6.4	6.5	6.5	6.6	6.4	6.6
37	6.6	6.6	6.6	6.6	6.7	6.5	6.7

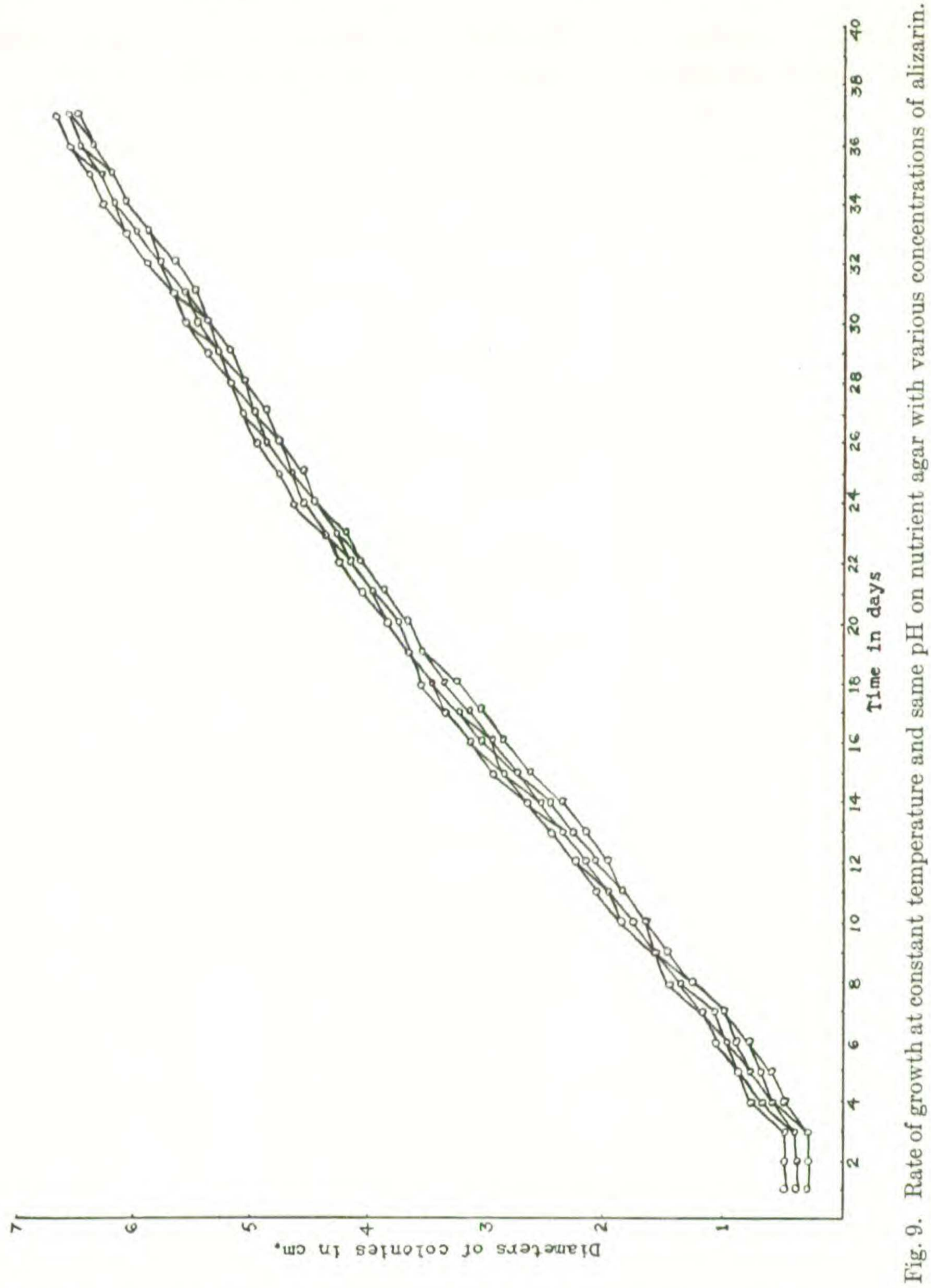


Fig. 9. Rate of growth at constant temperature and same pH on nutrient agar with various concentrations of alizarin.

showed that the rate was practically the same in each case, and if the diameters of the colonies in cm. were plotted against the time in days, for each concentration of the dye, one would find this to be the case. This is illustrated in fig. 9, the growth rate for

alizarin, results representative of these dyes. The differences in the curves are within the range of error, so that in general we may

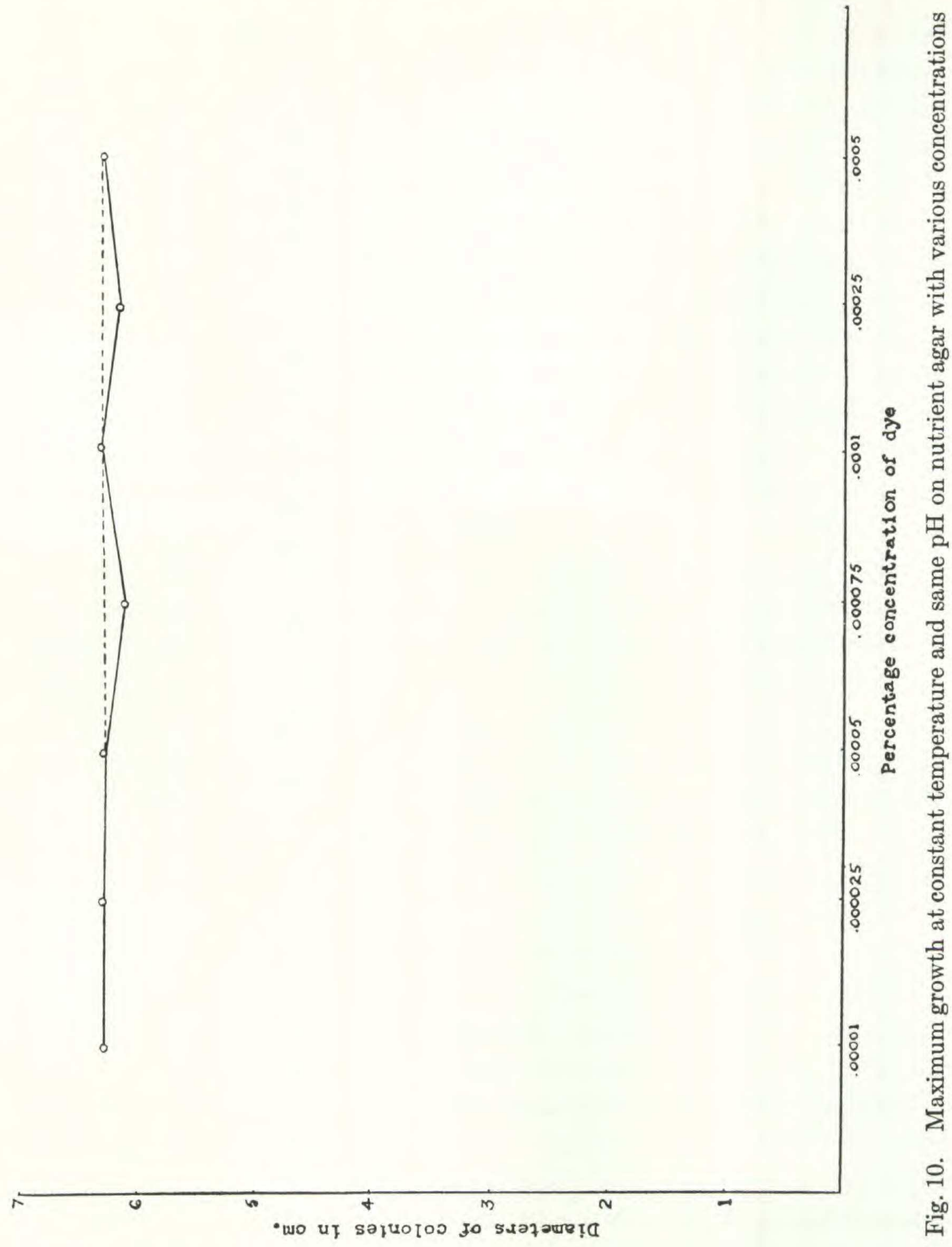


Fig. 10. Maximum growth at constant temperature and same pH on nutrient agar with various concentrations of alizarin.

say that the growth rate is the same. Except for the initial lag and a slight curve due perhaps to utilization of nutrient materials

or drying of the agar, the line is about straight. When the maximum points of growth for each concentration is plotted, that is, diameter of colony against percentage concentration of dye (fig. 10), it is found that a straight line results, with perhaps some excusable error. In other words, growth is identical for all the concentrations of dye, at least in the observations made here.

In dealing with the other dyes, as crystal violet (table v), methylene blue (table VI), and eosine B (table VII), a striking difference in growth was observed. The cultures of crystal violet grown under the same conditions as those above presented a growth which was proportional, within a range of limits, to the concentration of the dye. Like results were found to be true for methylene blue, but not quite so well marked.

Colony measurements were made daily, and, as may be seen in table v, the growth was inhibited in the higher concentrations to the point of complete cessation. Along with the measurements, microscopic examinations were also made, and it was found that on the media of higher concentration of dye there was a corresponding decrease in production of reproductive parts. In other words, the number of asci with the ascospores gradually diminished on the increasing concentration of dye media as compared with the normal growth. A well-marked feature was the development of thick walls in the hyphae, as may be seen in fig. 8 which illustrates variously thick-walled swellings, chlamydospores and hyphae, taken from a culture of .00025 concentration, forty days after inoculation. The cellular material stained heavily, and showed granular cytoplasm and a reduction in the number of nuclear divisions. An increase in the number of swellings, cellular contents, and thickness of walls, as well as the number of chlamydospores and terminal hypnospores, is a condition which is usually associated with unfavorable phenomena. Similar characteristics were present approximately three months later. Macroscopically, the colonies on the higher concentration of crystal violet showed a growth upwards on the inoculum and not until 15 days after inoculation did any growth appear on the agar and then very little. In the case of methylene blue, however, growth was flat and took on a blue color very soon after inoculation.

TABLE V

MEAN DIAMETERS IN CENTIMETERS OF COLONIES GROWN IN VARIOUS CONCENTRATIONS OF CRYSTAL VIOLET, AT A TEMPERATURE OF APPROXIMATELY 22° C.

Nutrient agar (product of Digestive Ferments Co.) pH 6.8

Days after inoculation	Percentage concentration of dye						
	.00001	.000025	.00005	.000075	.0001	.00025	.0005
1	0.3	0.2	0.3	0.2	0.4	0.3	0.3
2	0.3	0.2	0.3	0.2	0.4	0.3	0.3
3	0.3	0.2	0.3	0.2	0.4	0.3	0.3
4	0.5	0.3	0.3	0.2	0.4	0.3	0*
5	0.7	0.5	0.4	0.3	0.4	0.3	0
6	0.9	0.6	0.5	0.3	0.4	0.3	0
7	1.0	0.8	0.5	0.4	0.4	0.3	0
8	1.2	0.9	0.6	0.4	0.4	0.3	0
9	1.5	1.1	0.6	0.4	0.4	0.3	0
10	1.7	1.3	0.6	0.4	0.5	0.3	0
11	1.9	1.4	0.6	0.4	0.5	0.3	0
12	2.1	1.6	0.6	0.4	0.6	0.3	0
13	2.3	1.8	0.7	0.5	0.6	0.3	0
14	2.5	2.0	0.7	0.5	0.6	0.3	0
15	2.9	2.1	0.7	0.5	0.6	0.4	0
16	3.0	2.2	0.8	0.6	0.6	0.4	0
17	3.2	2.3	0.8	0.6	0.7	0.4	0
18	3.4	2.5	0.8	0.6	0.7	0.4	0
19	3.6	2.7	0.8	0.7	0.7	0.4	0
20	3.8	2.9	0.9	0.7	0.7	0.5	0
21	3.9	3.0	0.9	0.7	0.7	0.5	0
22	4.1	3.1	0.9	0.7	0.8	0.5	0
23	4.3	3.2	1.0	0.8	0.8	0	0
24	4.4	3.4	1.0	0.8	0.8	0	0
25	4.6	3.5	1.1	0.8	0	0	0
26	4.7	3.6	1.2	0.8	0	0	0
27	4.9	3.7	1.4	0.8	0	0	0
28	5.0	3.9	1.5	0.9	0	0	0
29	5.2	4.1	1.6	0.9	0	0	0
30	5.3	4.2	1.7	1.0	0	0	0
31	5.5	4.4	1.8	1.0	0	0	0
32	5.7	4.6	1.9	1.0	0	0	0
33	5.9	4.7	2.0	1.1	0	0	0
34	6.1	4.9	2.1	1.1	0	0	0
35	6.2	5.1	2.2	1.1	0	0	0
36	6.4	5.2	2.3	1.1	0	0	0
37	6.6	5.3	2.4	1.2	0	0	0

*0 indicates no growth

TABLE VI

MEAN DIAMETERS IN CENTIMETERS OF COLONIES GROWN IN VARIOUS CONCENTRATIONS OF METHYLENE BLUE, AT A TEMPERATURE OF APPROXIMATELY 22° C.

Nutrient agar (product of Digestive Ferments Co.) pH 6.8

Days after inoculation	Percentage concentration of dye						
	.00001	.000025	.00005	.000075	.0001	.00025	.0005
1	0.3	0.3	0.3	0.3	0.2	0.4	0.3
2	0.3	0.3	0.3	0.3	0.2	0.4	0.3
3	0.3	0.3	0.3	0.3	0.2	0.4	0.3
4	0.5	0.4	0.3	0.3	0.2	0.4	0.3
5	0.7	0.5	0.3	0.3	0.2	0.4	0.3
6	0.9	0.6	0.4	0.4	0.2	0.4	0.3
7	1.1	0.8	0.5	0.4	0.3	0.4	0.3
8	1.4	1.0	0.5	0.4	0.3	0.5	0.4
9	1.6	1.1	0.6	0.4	0.4	0.5	0.4
10	1.8	1.3	0.7	0.5	0.4	0.5	0.4
11	2.0	1.5	0.9	0.6	0.5	0.5	0.4
12	2.2	1.7	1.0	0.7	0.5	0.5	0.5
13	2.4	1.8	1.1	0.8	0.6	0.5	0.5
14	2.6	2.0	1.2	0.8	0.6	0.5	0.5
15	2.8	2.1	1.3	0.8	0.6	0.5	0.5
16	2.9	2.3	1.5	0.9	0.7	0.6	0.5
17	3.1	2.5	1.6	0.9	0.7	0.6	0.6
18	3.4	2.7	1.7	0.9	0.7	0.6	0.6
19	3.6	2.8	1.8	1.0	0.8	0.6	0.6
20	3.8	3.0	1.9	1.0	0.8	0.6	0.7
21	4.0	3.2	2.0	1.0	0.8	0.7	0.7
22	4.1	3.4	2.1	1.1	0.9	0.7	0.7
23	4.4	3.5	2.2	1.1	0.9	0.7	0.8
24	4.6	3.6	2.4	1.2	1.0	0.7	0.8
25	4.7	3.8	2.4	1.3	1.0	0.7	0.8
26	4.9	4.0	2.6	1.3	1.1	0.8	0.8
27	5.1	4.2	2.7	1.4	1.1	0.8	0.8
28	5.2	4.3	2.9	1.6	1.2	0.8	0.8
29	5.4	4.4	3.0	1.7	1.2	0.9	0.8
30	5.6	4.6	3.2	1.8	1.3	0.9	0.9
31	5.7	4.7	3.3	1.8	1.3	0.9	0.9
32	5.9	4.9	3.5	1.9	1.4	0.9	0.9
33	6.1	5.1	3.6	2.1	1.5	1.0	0.9
34	6.3	5.2	3.8	2.2	1.6	1.0	0.9
35	6.5	5.3	4.0	2.3	1.7	1.1	0.9
36	6.7	5.4	4.1	2.4	1.8	1.1	0.9
37	6.8	5.5	4.2	2.5	1.9	1.2	0.9

TABLE VII

MEAN DIAMETERS IN CENTIMETERS OF COLONIES GROWN IN VARIOUS CONCENTRATIONS OF EOSINE B, AT A TEMPERATURE OF APPROXIMATELY 22° C.

Nutrient agar (product of Digestive Ferments Co.) pH 6.8

Days after inoculation	Percentage concentration of dye						
	.00001	.000025	.00005	.000075	.0001	.00025	.0005
1	0.3	0.2	0.3	0.3	0.5	0.4	0.5
2	0.3	0.2	0.3	0.3	0.5	0.4	0.5
3	0.3	0.2	0.3	0.3	0.5	0.4	0.5
4	0.4	0.4	0.4	0.6	0.7	0.4	0.7
5	0.6	0.5	0.6	0.7	0.9	0.6	0.7
6	0.7	0.6	0.7	0.8	1.0	0.7	0.8
7	0.9	0.9	0.9	1.0	1.2	0.9	1.0
8	1.2	1.2	1.2	1.3	1.4	1.1	1.3
9	1.3	1.3	1.3	1.4	1.6	1.3	1.4
10	1.5	1.5	1.5	1.6	1.8	1.5	1.6
11	1.6	1.7	1.7	1.8	2.0	1.8	1.8
12	1.9	1.9	1.9	2.0	2.2	2.0	2.1
13	2.1	2.1	2.1	2.2	2.4	2.2	2.3
14	2.3	2.3	2.3	2.5	2.6	2.4	2.5
15	2.5	2.5	2.5	2.6	2.9	2.6	2.7
16	2.7	2.7	2.7	2.9	3.1	2.8	2.9
17	2.9	2.9	2.9	3.1	3.3	3.1	3.1
18	3.1	3.1	3.2	3.2	3.5	3.2	3.2
19	3.3	3.3	3.3	3.4	3.6	3.4	3.2
20	3.5	3.4	3.5	3.5	3.8	3.5	3.2
21	3.6	3.6	3.7	3.7	3.9	3.7	0
22	3.8	3.8	3.8	3.9	4.1	3.8	0
23	3.9	4.0	4.0	4.0	4.2	3.9	0
24	4.1	4.2	4.2	4.2	4.3	4.1	0
25	4.2	4.4	4.3	4.3	4.5	4.3	0
26	4.4	4.5	4.5	4.5	4.6	4.5	0
27	4.6	4.7	4.7	4.6	4.8	4.6	0
28	4.7	4.8	4.8	4.8	4.9	4.8	0
29	4.9	5.0	5.0	5.0	5.1	5.0	0
30	5.1	5.2	5.2	5.1	5.2	5.1	0
31	5.3	5.3	5.3	5.3	5.4	5.3	0
32	5.4	5.5	5.5	5.5	5.5	5.5	0
33	5.6	5.7	5.7	5.6	5.7	5.5	0
34	5.7	5.8	5.8	5.8	5.8	0	0
35	5.9	6.0	6.0	5.9	6.0	0	0
36	6.0	6.1	6.1	6.1	6.1	0	0
37	6.2	6.3	6.3	6.2	6.2	0	0

The same microscopical features were also observed in the case of methylene blue, but not quite so outstanding. The rates of growth for both crystal violet and methylene blue, as seen in figs. 11 and 13 respectively, showed a decrease with a more marked sudden drop in the case of crystal violet. These may perhaps be better illustrated in figs. 12 and 14, both being corrected for the inoculum so that the curves represent the total growth at each concentration of the dye for the period denoted. The growth on crystal violet showed a sudden decrease which comes to zero at a concentration of .0005. In the case of methylene blue, however, the decrease was gradual, and the curve may be said to be practically straight.

These results are therefore in accord with the genesistasis beliefs of Churchman or the "bacteriostatic properties," and may be further substantiated by the observation that the inoculum of the crystal violet medium of concentration .0005, which had shown no growth over a period of four months, was transferred to fresh medium and developed into a normal and healthy culture.

A phenomenon which is difficult to explain in the light of this work is the fact that in concentrations of .0001, .00025 and .0005, where there had been very little or no growth, a renewed activity occurred approximately five months after inoculation, in flasks where there had been a rather large amount of agar which had not dried out. A possible explanation may be perhaps that the organism had become accustomed to the dye and, there being enough nutrient in the medium, it began to grow, developing hyphae from the chlamydospores which had been dormant. A microscopic examination of these renewed viability cultures showed many chlamydospores (round) and thick-walled hyphae still present. A chemical interpretation of this phenomenon has as yet not been postulated and will perhaps require much more work and a better understanding of the reaction.

In the case of eosine B, growth was normal on all concentrations up to the nineteenth day, as seen in table VII, when growth actually ceased in the medium of the highest concentration of the dye (.0005). This was followed by a cessation in growth on the thirty-fourth day for the concentration .00025. This phenomenon has been noticed and reported in previous papers by the

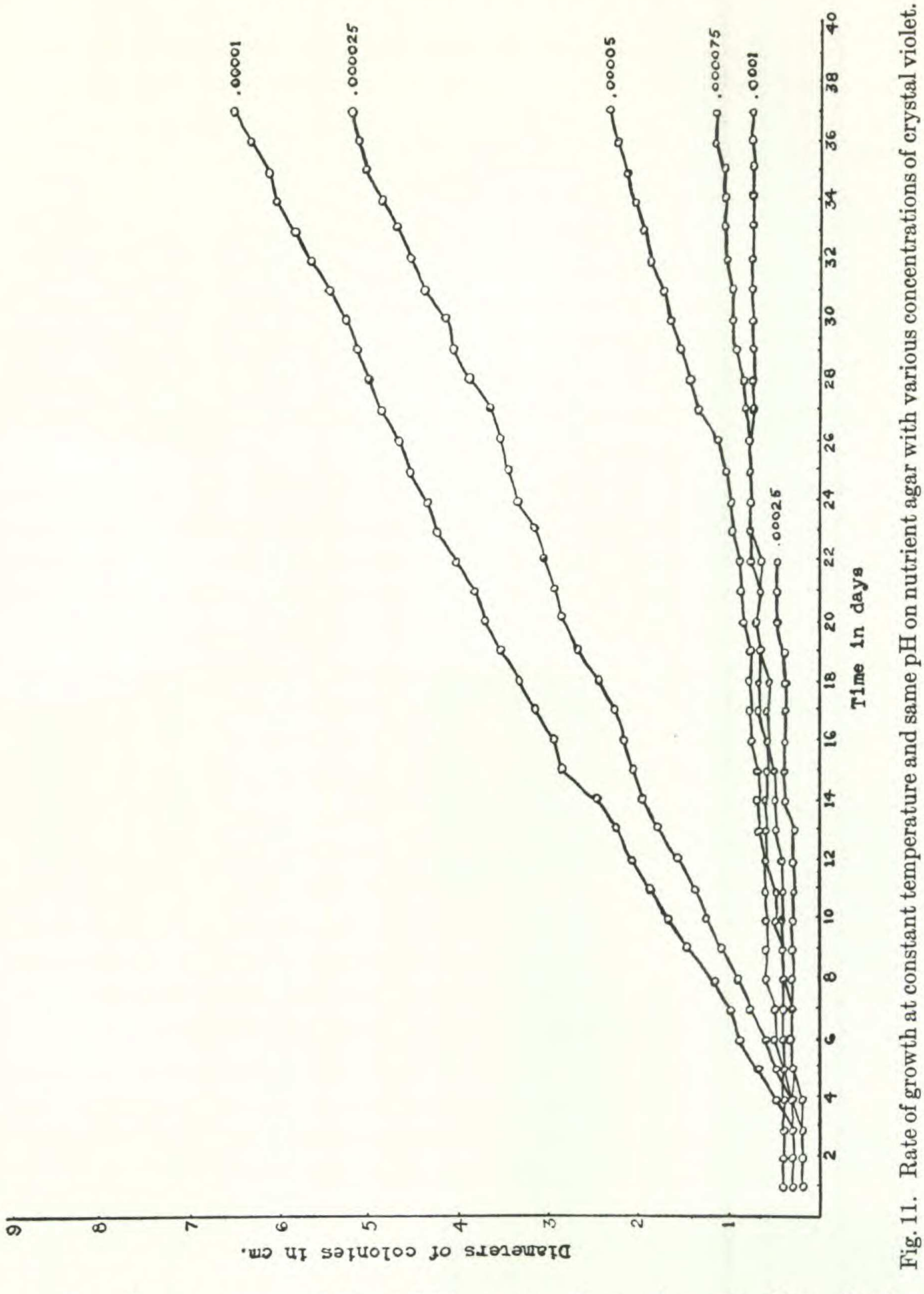


Fig. 11. Rate of growth at constant temperature and same pH on nutrient agar with various concentrations of crystal violet.

author for eosine-methylene-blue agar where the mycelium had taken on a pink to blue color and ceased growing. These cultures when transplanted to fresh agar grew just as luxuriantly as

though they had never been affected. What may have happened is that the dye, being acid, may have been absorbed by the fungus,

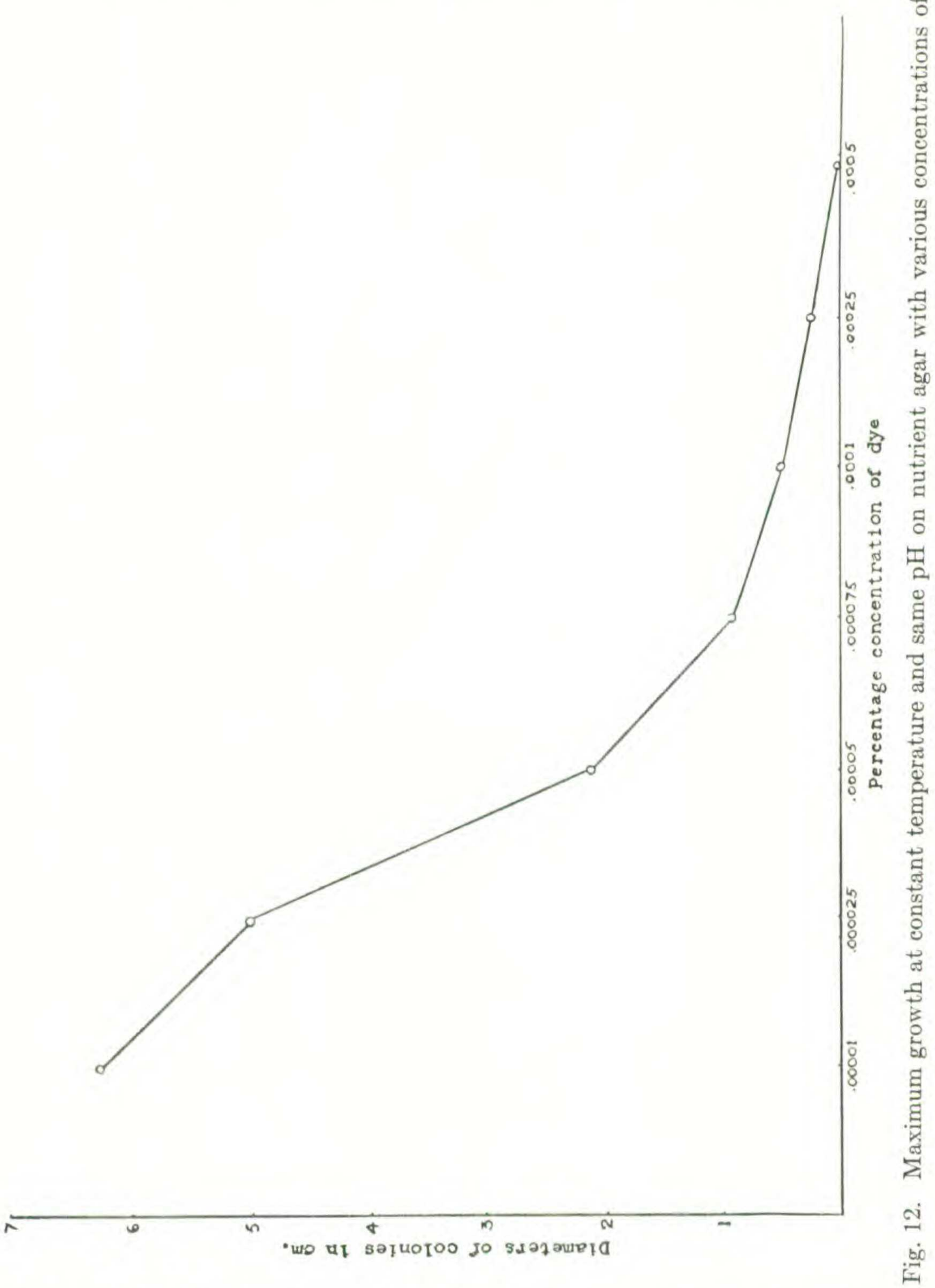


Fig. 12. Maximum growth at constant temperature and same pH on nutrient agar with various concentrations of crystal violet.

as evidenced by the color of the mycelium, and was strong enough to counteract the metabolic activities of the cell. It would seem

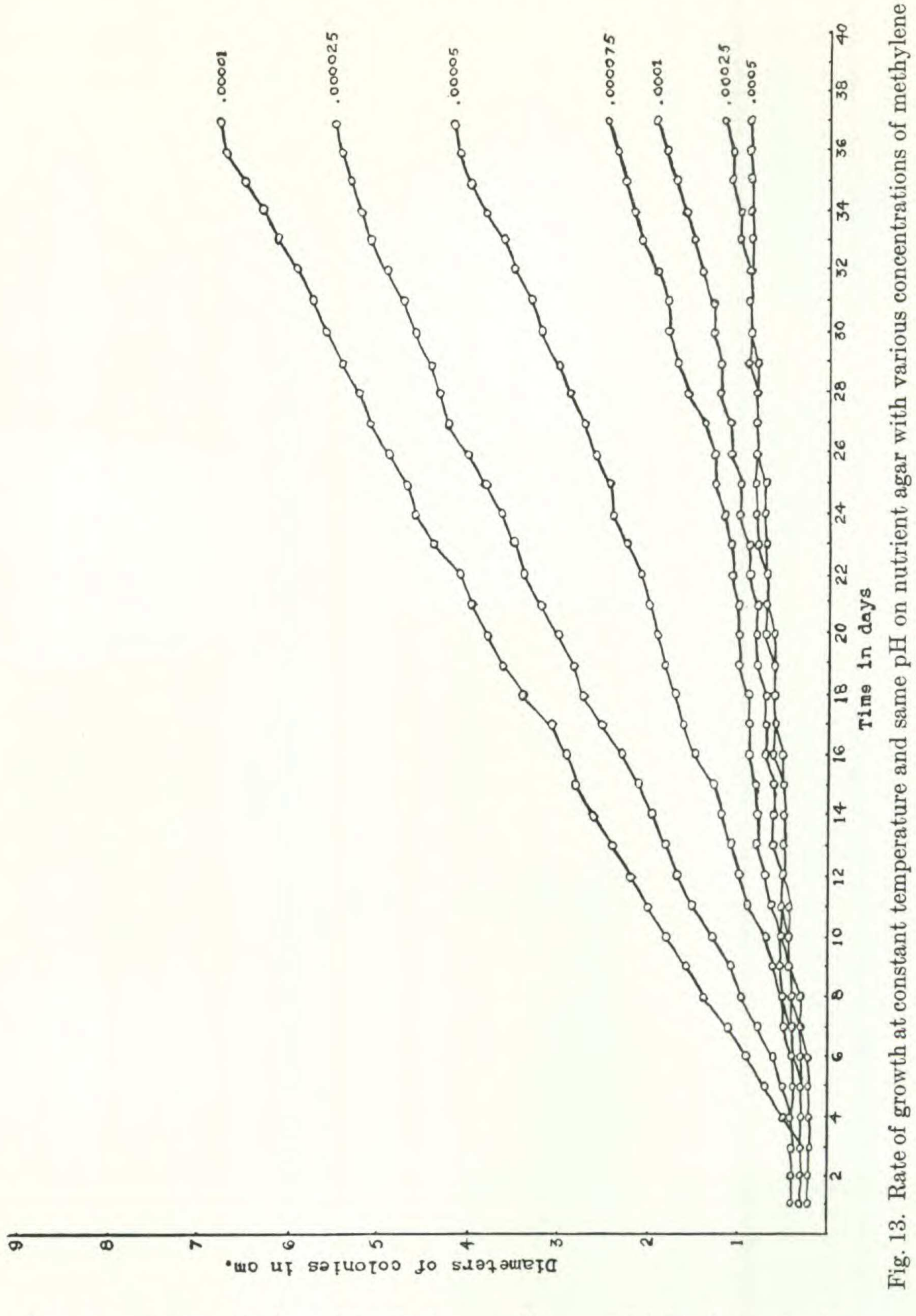


Fig. 13. Rate of growth at constant temperature and same pH on nutrient agar with various concentrations of methylene blue.

from a study of the pH of the medium, which changes from a slightly acid to alkaline in normal growth, that this could be detected. However, the medium was already covered with

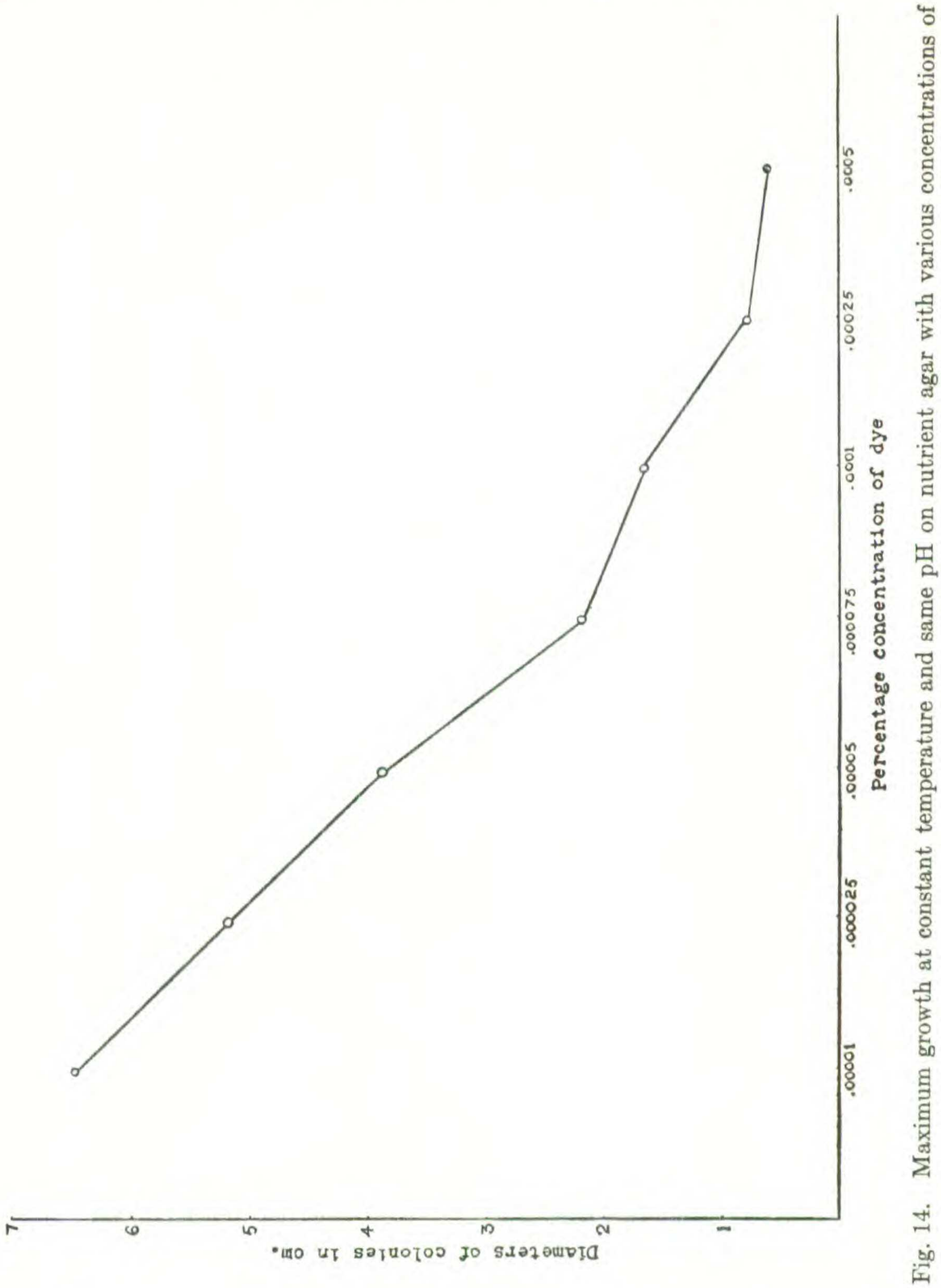


Fig. 14. Maximum growth at constant temperature and same pH on nutrient agar with various concentrations of methylene blue.

sufficient growth to cause an alkaline condition, so that no direct evidence could be obtained from that direction. There are no definite facts to uphold this belief, and the statement should be considered more as a conjecture for the present. There have

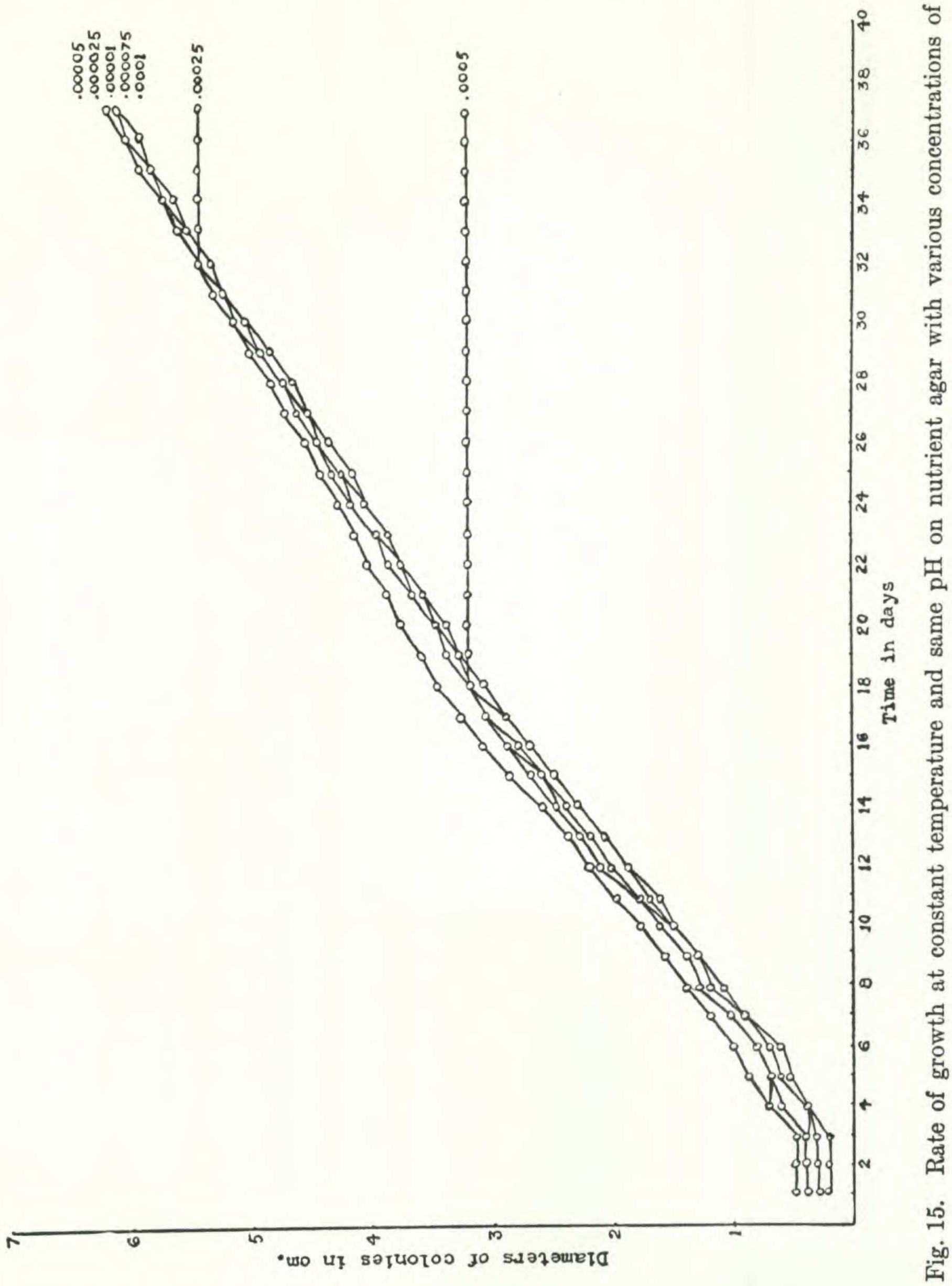


Fig. 15. Rate of growth at constant temperature and same pH on nutrient agar with various concentrations of eosine B.

been many theories proposed, but none have had any final acceptance.

The action of eosine B on the organism is illustrated in figs. 15–16. The former shows the rate of growth of the cultures on

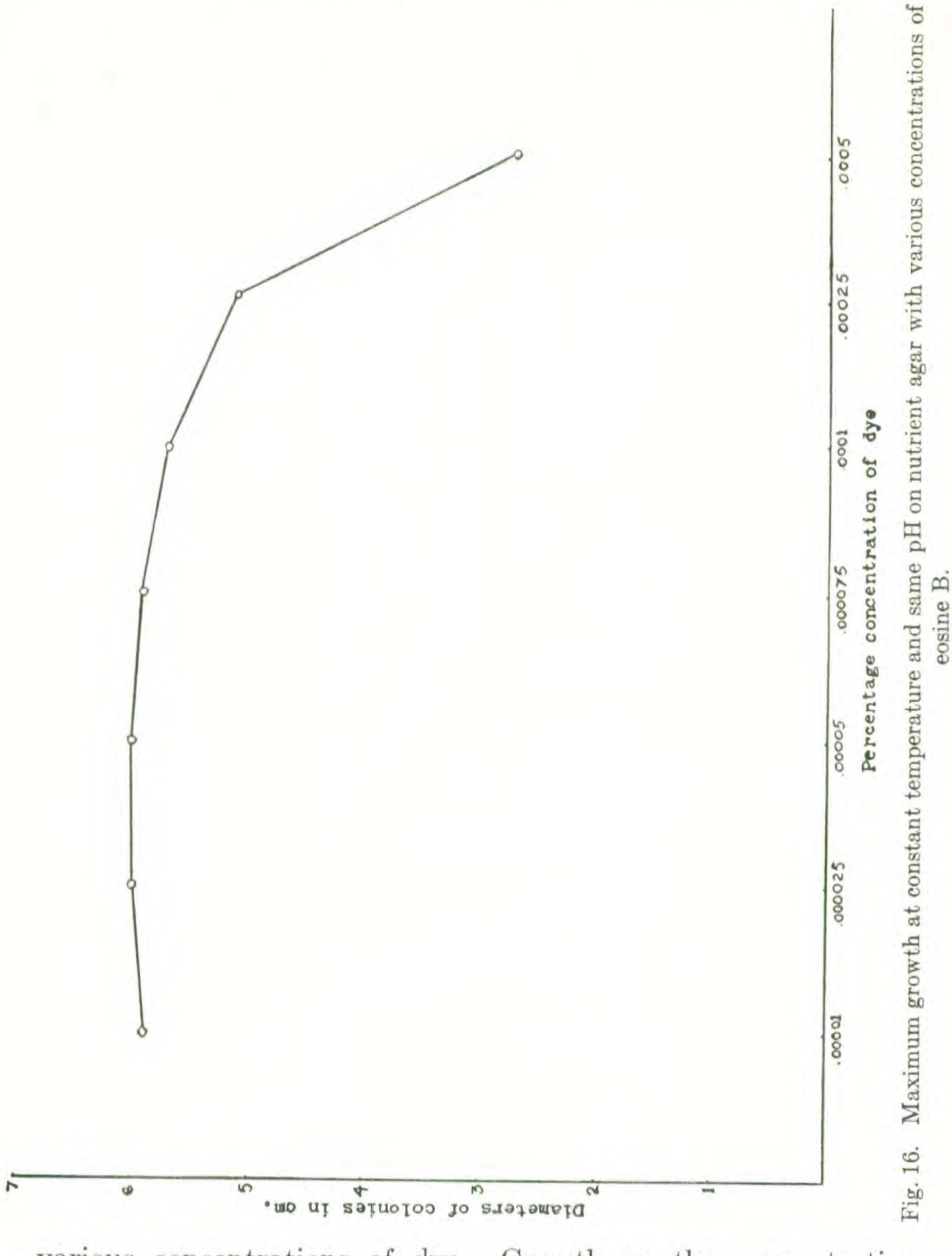


Fig. 16. Maximum growth at constant temperature and same pH on nutrient agar with various concentrations of eosine B.

various concentrations of dye. Growth on the concentration .0005 was inhibited on the nineteenth day, and that on the concentration .00025, on the thirty-fourth day. Fig. 16 represents the maximal points of growth corrected for the inoculum. The inhibition of growth is clearly distinguishable here.

A further phenomenon is noted here, not because it was absolutely specific for this experiment but because it had appeared most frequently on crystal violet media of concentrations .0001 and .00025. This is the formation of crystals in the media, appearing to emanate from the growing zone or peripheral region of the colony and found in conditions which have not been very favorable towards growth. They have occurred on beef extract agar of pH 7.2, 7.0, and 6.8. No crystals of this sort were found on media where there was no growth or where growth was very luxuriant. They appear to resemble very closely those of ammonium magnesium phosphate ($NH_4MgPO_4.6\ H_2O$) as was reported also by Scudder ('26). They were insoluble in cold water, completely and immediately soluble in hydrochloric acid, and were reprecipitated with sodium hydroxide, tests which are applied to crystals of the above composition. They were apparently the result of fungal activity, and Scudder has associated this condition with bacteria which "do not ferment the ordinary carbohydrates and intensify the alkalinity of the medium rapidly," a fact which has been clearly demonstrated in the carbohydrate reactions carried out in this paper.

Animal Inoculations

From time to time papers appear in the literature pointing out that some particular organism which had been kept in culture over a long period and then inoculated in experimental animals, had retained its pathogenic properties. On the other hand, it is quite well known that this property may be lost on subculturing. It is also known that by growth on certain media at a specific temperature, toxicity may be retained over a long time. It has also been found that certain microorganisms may be made to regain lost toxic powers by repeated inoculations.

In order to determine whether *E. capsulatus* had undergone any such changes since it was isolated in September, 1928, and kept in culture at different temperatures, on different media, and at various hydrogen-ion concentrations, several animals were inoculated with a twelve-day-old suspension of the organism in 10-cc. physiological saline solution. The culture was stirred up thoroughly with a sterile needle to eliminate the clumps.

The animals and inoculations were as follows:

Rabbits: Two females—intracerebral (0.2 cc.) and intravenous (0.5 cc.). Two males—subcutaneous (2 cc.) and intratesticular (1 cc.).

Guinea-pigs: Two, one male and one female—intracerebral (0.2 cc.). Two males—subcutaneous (1.5 cc.) and intratesticular (1 cc.).

Mice: Two, one male and one female—intracerebral (0.2 cc.). One male—subcutaneous (1 cc.) and intratesticular (0.5 cc.).

The results of these inoculations were that one of the rabbits receiving a subcutaneous and intratesticular inoculation died twenty-three days later. Autopsy revealed no infection or lesion, except an intestinal obstruction. Three mice, healthy, with no loss of weight or appetite, were killed fifty days after inoculation. The organs were completely negative. Cultures of the heart's blood also negative. The rabbits and guinea-pig were healthy, with no loss of weight, three months after inoculation. The remaining mouse, with an intracerebral injection, died sixty-two days after inoculation as a result of pneumonia. Autopsy showed no complication of the other organs. Cultures of infected lung tissue and brain negative for *E. capsulatus*, the lung in a hemorrhagic condition.

It may be concluded, therefore, that the organism had lost its power to infect after four years' growth on artificial substrates. That it failed to produce a toxic action in an animal may be attributed to the changed environment and habitat of the fungus. It has been pointed out previously that the optimum temperature has been reduced from 37.5° C. to approximately 25° C. In addition, experimental data has shown that growth at the former temperature was very slow and slight, with complete cessation and death after a short time. It is very possible that the organism was killed here, as in the *in vitro* experiments, as a result of the body temperature of the experimental animals. A further point of interest is the condition of aerobiosis as found here. When freshly isolated, the yeast-cells of *E. capsulatus* or *E. dermatitidis* are able to live in a condition of facultative aerobiosis, that is, with or without oxygen, favoring the presence of air. With continued cultivation on artificial media, the organism

adapts itself to a saprophytic form of life and as a result becomes a strict aerobe, requiring oxygen for its existence. This change to strict aerobiosis is an important factor, since, by inoculation in the animal body, the organism is forced into an aerobic condition which it cannot endure and, as probably happened here, it is killed for lack of oxygen.

There are probably several other factors which may account for this failure of the organism to cause infection, but what has been said above accounts for the chief difficulties.

Summary and Conclusions

1. A review of the case histories from which the organisms *Endomyces capsulatus*, *E. capsulatus* var. *isabellinus*, *E. dermatitidis*, and the Austrian fungus were isolated are given.

2. A study of the organism shows that it has two life cycles: one in the parasitized host as a budding yeast-cell with the chromatin material spread throughout the cell; and the other as a perfect Ascomycete, reproducing by the development of 8-spored asci resulting from a sexual act which may be either hetero- or isogamy or have become reduced to parthenogamy.

3. No definite form of mitosis is demonstrable; instead conditions similar to those described as amitosis are recorded.

4. Cellular contents, as volutin, glycogen, vacuoles, chondriosomes, and fat or lipoidal substances, are demonstrated in *E. capsulatus* by various methods.

5. The cultural work is described in detail and includes descriptions of the organism on 29 different media, showing that hydrogen-ion concentration may influence growth as expressed by thin hyphae and numerous conidia on acid media and thick-walled, shorter cells on alkaline media.

6. After four years of subculturing an optimum temperature of 25° C. is determined for the organism, with no growth occurring above 37° C. or below 8.0° C.

7. An optimum pH of 7.4 is determined for *E. capsulatus*, with no growth occurring at a pH greater than 9.3 or less than 3.3.

8. The carbohydrates used in this work require a variation in time for the production of alkalinity. No acid or gas is produced. Hydrogen-ion measurements show a decrease from

the initial pH of 6.8 to an end point of approximately 8.1, which is accounted for by the production of ammonia and probably hexone bases.

9. Under proper conditions, indol and skatol reactions are present.

10. In the yeast form *E. capsulatus* may show facultative anaerobiosis, but growth on an artificial substrate converts the organism into a strict aerobe.

11. Nitrate reduction and hydrogen sulphide production yield indefinite results.

12. Gelatine is slowly liquefied after 30 days.

13. Light, either white or red, has no effect on the organism as compared with that of dark or diffuse daylight and total darkness.

14. Several dyes, orange G, Sudan III, alizarin, neutral red, safranine A, light green SF, basic fuchsin, aniline blue, phenol red, and haematoxylin, in concentrations of .00001, .000025, .00005, .000075, .0001, .00025, .0005, when incorporated in an agar medium have no effect on growth. Crystal violet and methylene blue in the same concentrations show a gradual decrease in growth, with complete cessation at a concentration of .0005 for crystal violet. Crystal violet showed a reduction in the number of nuclear divisions, sexual fusions, and production of asci, with an accompanying enlargement of the cells, development of thick walls, secretion of granular material in the cytoplasm, conditions associated with the formation of chlamydospores under unfavorable conditions. Eosine B has an effect on growth in high concentrations.

15. Animal inoculations for pathogenicity were negative after subculturing for a period of approximately four years.

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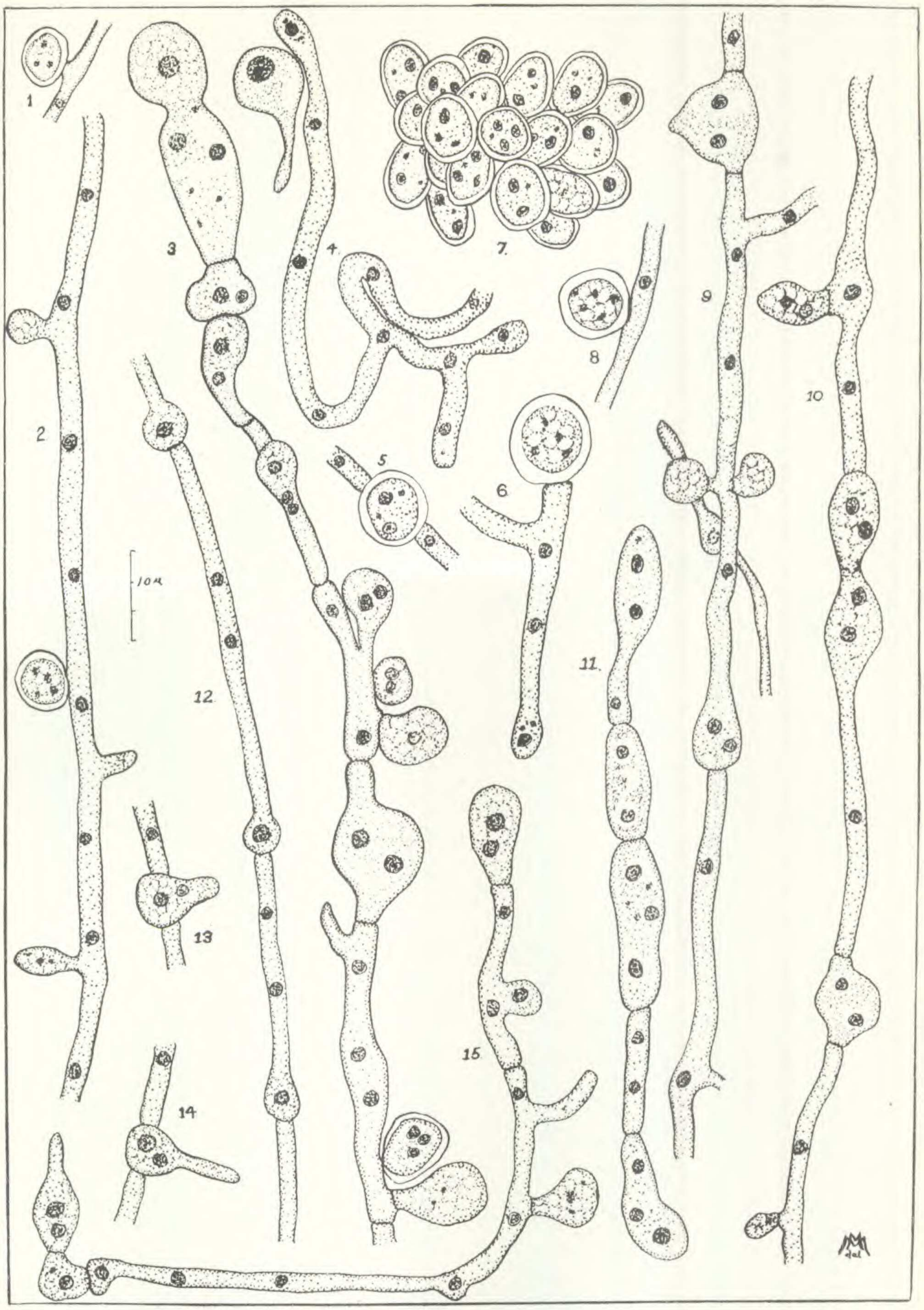
Explanation of Plate

PLATE 16

All figures drawn at a magnification of × 1440 with the aid of a camera lucida.

Fig. 1. Thick-walled cell on glycerine agar.
Fig. 2. Young mycelium.
Fig. 3. Mature mycelium with conidia, terminal hypnospores, and oidia.
Fig. 4. Mycelium on blood agar.
Fig. 5. Intercalary chlamydospore.
Fig. 6. Terminal hypnospore.
Fig. 7. Group of thick-walled cells on bacto-peptone plus glycerine.
Fig. 8. Thick-walled cell on corn-meal agar.
Fig. 9. Mycelium with racquet hyphae, conidia, and intercalary chlamydospore.
Fig. 10. Mycelium grown on calcium carbonate medium.
Fig. 11. Mycelium on glycerine agar.
Fig. 12. Racquet mycelium on Sabouraud's agar.
Fig. 13. Endo-chlamydospore.
Fig. 14. Growth of fig. 13.
Fig. 15. Mycelium with conidia, young developing hyphal branch, and hypnospores on beef extract agar.



MOORE—ENDOMYCES CAPSULATUS

Explanation of Plate

PLATE 17

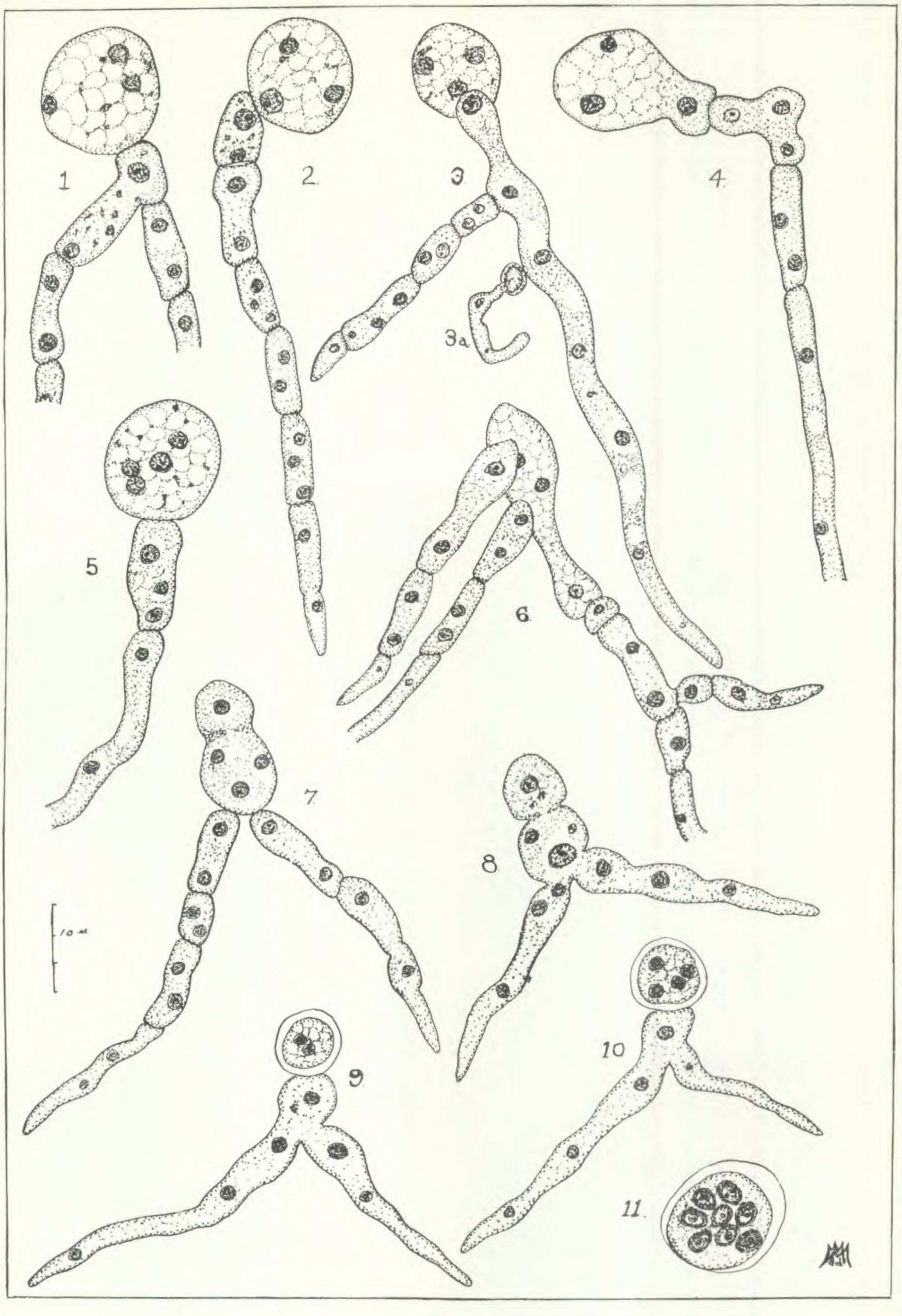
All drawings made from a hanging-drop culture of bacto-peptone plus glycerine, with the aid of a camera lucida at × 1440.

Figs. 1–5. Chlamydospores and young hyphae.

Fig. 3a. Young germinating spore.

Fig. 6. Group of germinating spores.

Figs. 7–11. Developmental series observed over a period of 3 days with the final formation of an ascus with 8 spores (fig. 11). Condition analogous to the Stoppel form of fertilization.



MOORE—ENDOMYCES CAPSULATUS

Explanation of Plate

PLATE 18

All figures drawn with the aid of a camera lucida at a magnification of × 1440.

Fig. 1. Hypha on malt extract agar showing chlamydospores.

Fig. 2. Terminal chlamydospore or ascogonial cell.

Fig. 3. Hyphal termination.

Figs. 4–5. Yeast-like cells with thick walls, grown on beef extract plus blood serum agar.

Fig. 6. Hypha with pyriform conidia, lateral chlamydospores on wort agar.

Fig. 7. Hyphae showing an abundance of conidia on Raulin's solution agar.

Fig. 8. Mycelium on wort agar.

Fig. 9. A spore which has germinated and produced a large lateral cell.

Fig. 10. Racquet mycelium with a large pyriform conidium.

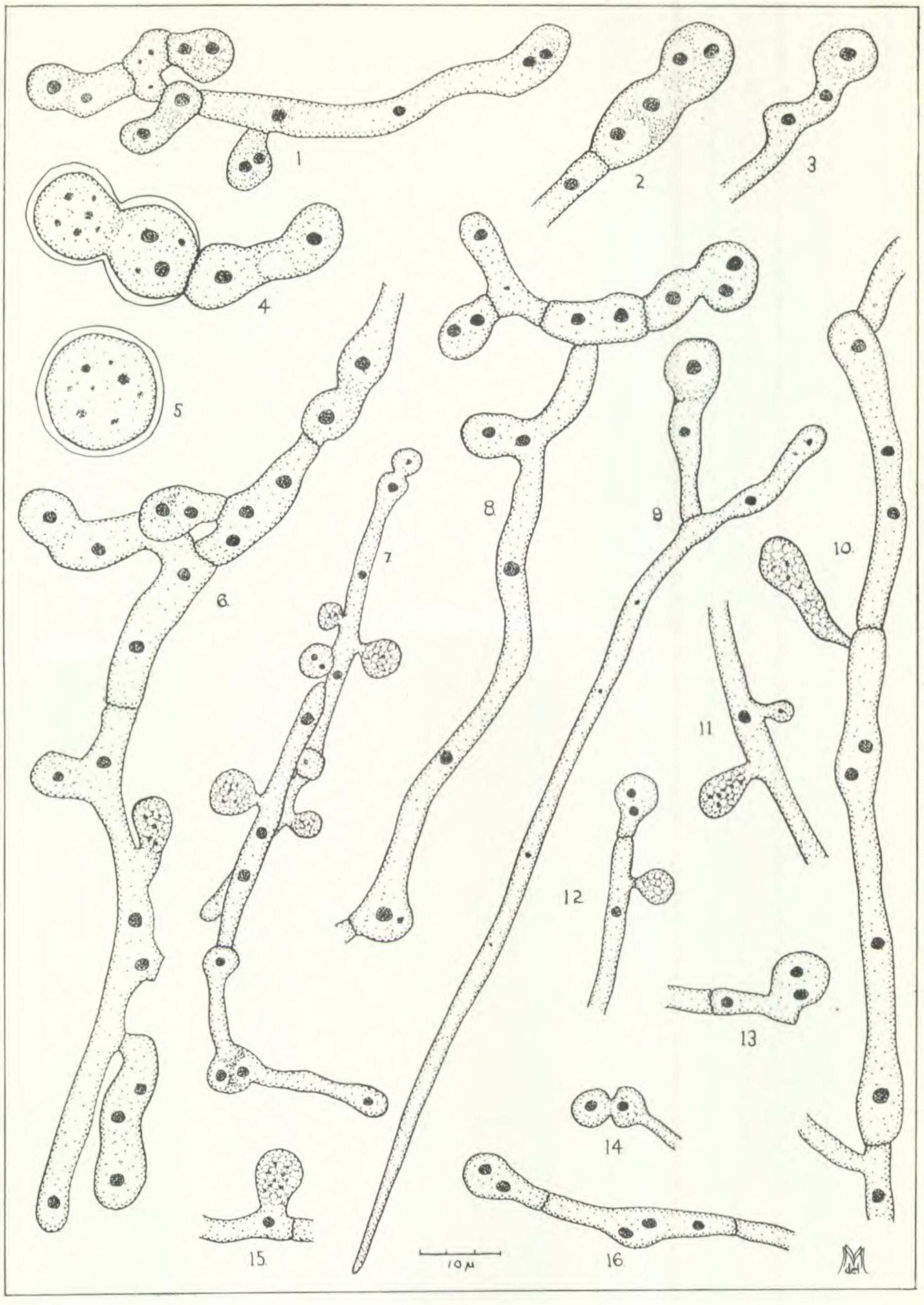
Fig. 11. Conidia on lactose agar.

Fig. 12. Terminal chlamydospore and lateral conidium or perhaps chlamydospore on beef extract plus blood serum agar.

Fig. 13. Lateral chlamydospore.

Figs. 14, 16. Cells of the above.

Fig. 15. Conidium on wort agar.



MOORE—ENDOMYCES CAPSULATUS

KAYNE, BOSTON

EXPLANATION OF PLATE

PLATE 19

All drawings made with the aid of a camera lucida at × 1440.

Figs. 1–4. Germination of spores on 2 per cent bacto-peptone plus 5 per cent meat extract.

Fig. 5. Pyriform, sessile conidium on Richards' solution agar.

Fig. 6. Ascus.

Fig. 7. Hyphae showing round conidia and an intercalary chlamydospore.

Fig. 8. Mycelium with chlamydospores and conidia on Raulin's solution agar.

Fig. 9. Bit of young hypha with a large chlamydospore on Richards' solution agar.

Fig. 10. Terminal hypnospore and a lateral chlamydospore on Raulin's solution agar.

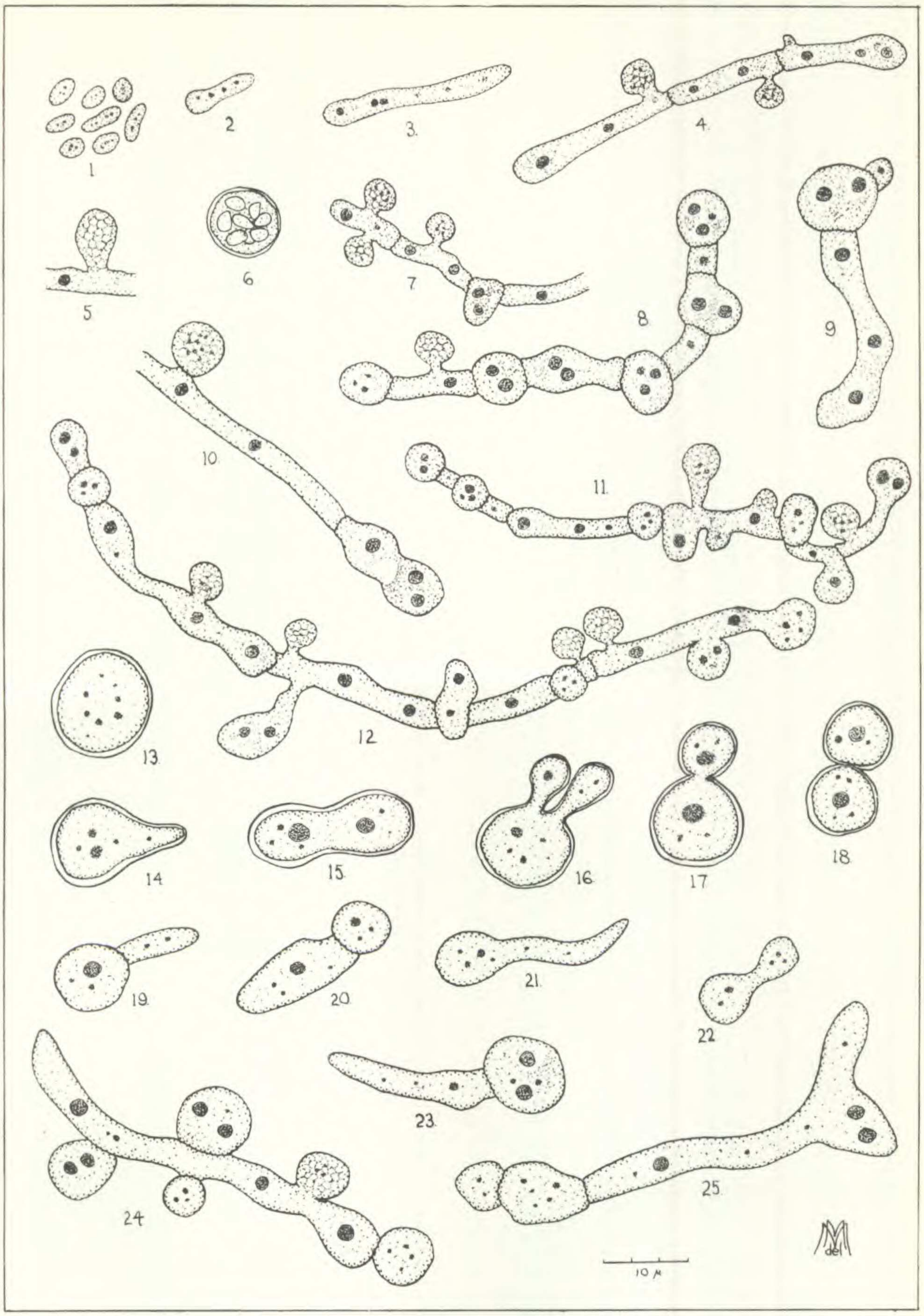
Fig. 11. Hypha with numerous pyriform and round conidia as well as chlamydospores.

Fig. 12. Hypha showing conidia, chlamydospores, and probably an ascogenous cell.

Figs. 13–25. Yeast-like cells and hyphae of the organism from the Austrian case on Sabouraud's agar.

Figs. 13–18. Showing budding.

Figs. 19–25. Formation of hyphae with conidia and chlamydospores.



MOORE—ENDOMYCES CAPSULATUS

EXPLANATION OF PLATE

PLATE 20

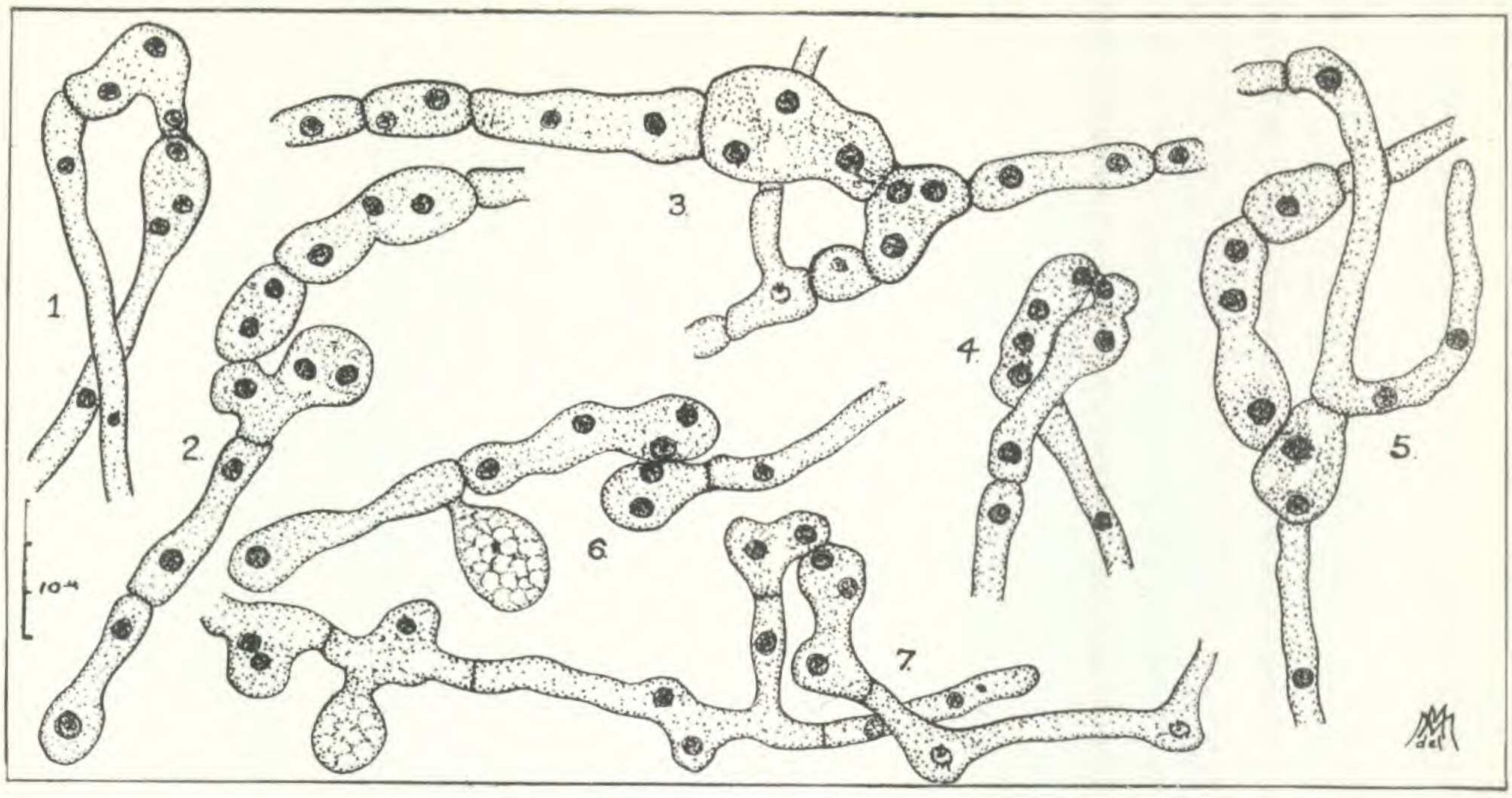
A

Forms of copulation drawn with the aid of a camera lucida at a magnification of × 1440.

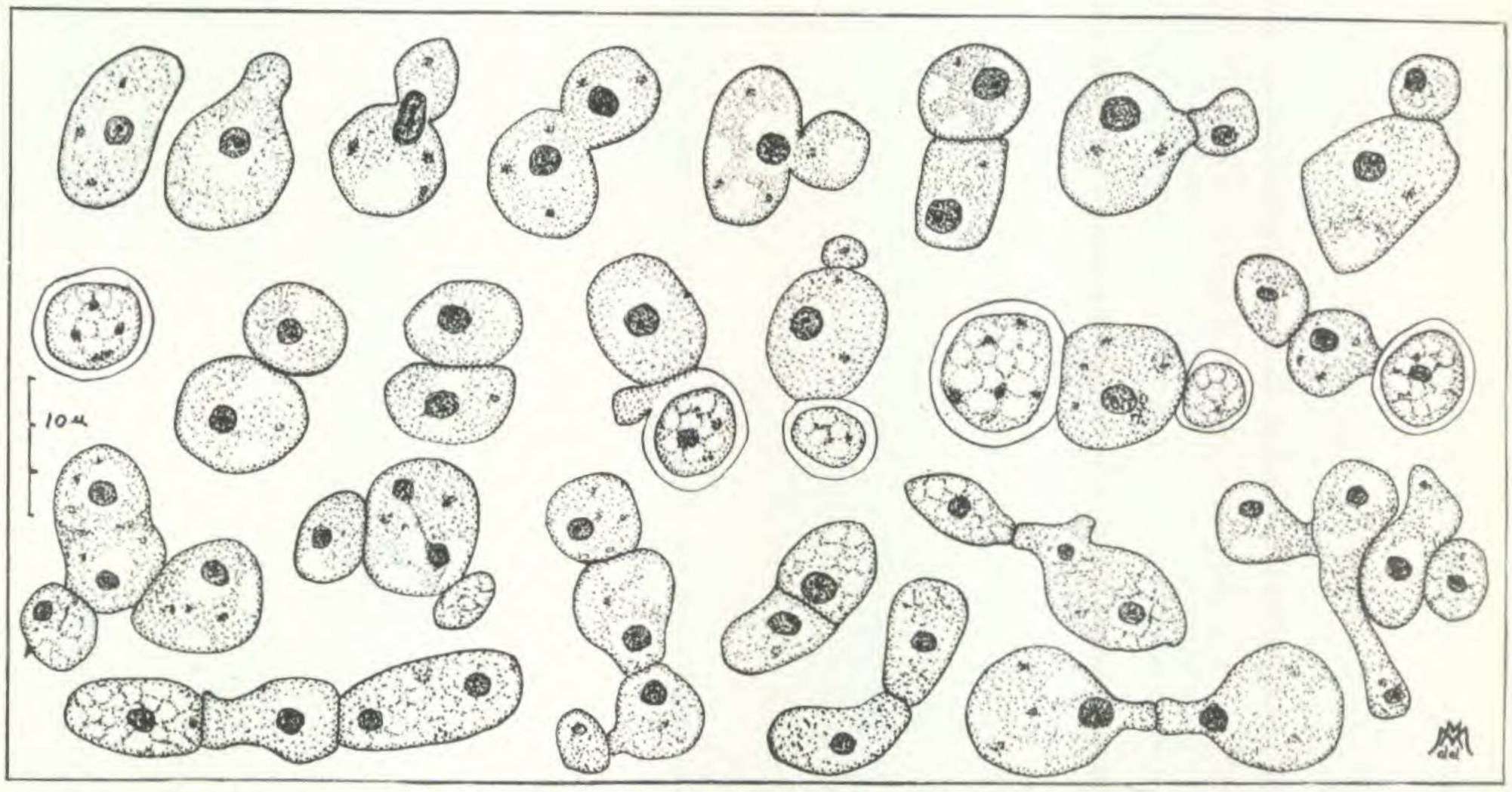
Fig. 1. Terminal copulation.
Fig. 2. Probably a form of copulation.
Fig. 3. Termino-lateral copulation.
Fig. 4. Same as fig. 1.
Fig. 5. Same as fig. 3.
Fig. 6. Lateral form of terminal copulation.
Fig. 7. Terminal copulation.

B

Cells observed on a medium which contained a high pH. Reversion to a yeast-like form × 1440.



A



B

MOORE—ENDOMYCES CAPSULATUS

COCKAYNE, BOSTON

EXPLANATION OF PLATE

PLATE 21

All drawings made at a magnification of × 1440 with the aid of a camera lucida.

Figs. 1–8. Yeast-like and budding cells on 2 per cent bacto-peptone (aqueous). Fresh isolation.

Figs. 9–11. Saccharomycetous cells developing mycelium in a hanging-drop culture of 2 per cent aqueous solution of bacto-peptone plus 5 per cent meat extract.

Figs. 12–13. Later stages of the above.

Fig. 14. Heterogamous copulation, premature stage on Sabouraud's agar.

Fig. 15. Heterogamous copulation seen at time of transfer of nuclear material, in hanging-drop of 2 per cent aqueous proteose peptone plus glycerine.

Fig. 16. Germinating spore on glycerine agar.

Fig. 17. Germinating spore developing hyphae on lactose agar.

Fig. 18. Developing ascus on Sabouraud's agar.

Fig. 19. Ascus showing 8 spores on nutrient agar.

Fig. 20. Racquet mycelium on lactose agar.

Fig. 21. Oidia-like cells formed in Sabouraud's broth.

Fig. 22. Ascus on potato-dextrose agar.

Fig. 23. Terminal hypnospore (chlamydospore) on glycerine agar.

Fig. 24. Large round chlamydospore with a thick wall on corn-meal agar.

Fig. 25. Section of a mycelium showing a thick-walled chlamydospore in a hanging-drop of 2 per cent bacto-peptone (aqueous).

Fig. 26. Mycelium with conidia on glycerine agar.

Fig. 27. Mycelium on eosine-methylene-blue agar.

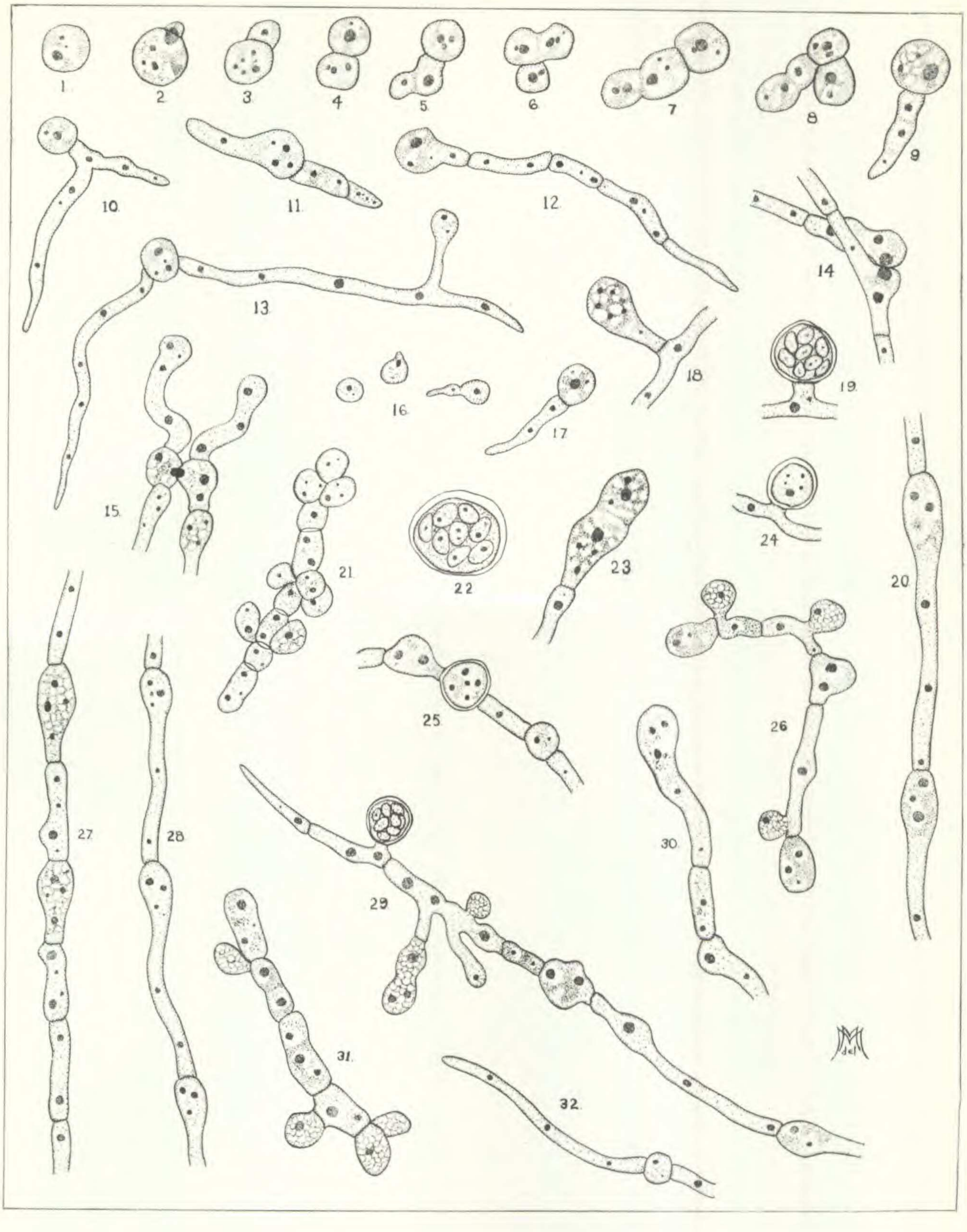
Fig. 28. Racquet mycelium on Sabouraud's agar.

Fig. 29. Mycelium showing racquet formation, conidia, hypnospores, and an 8-spored ascus, on glycerine agar.

Fig. 30. Terminal hypnospore on 2 per cent proteose peptone plus glycerine.

Fig. 31. Monilia-like growth in nutrient broth.

Fig. 32. Young hypha, 2 per cent bacto-peptone hanging-drop culture.



MOORE—ENDOMYCES CAPSULATUS

VAR. ISABELLINUS

KAYNE, BOSTON

Explanation of Plate

PLATE 22

All drawings made with the aid of a camera lucida and high oil-immersion magnification. × 2300.

All cultures grown on glycerine agar.

Figs. 1–3. Germinating spores. Fixed with Hermann's and stained with iron haematoxylin.

Fig. 4. Young hypha prior to septum formation showing many nuclei, some dividing.

Fig. 5. Hypha with septa formed.

Figs. 6–7. Antheridia with dividing nuclei. Fixed with Benda's and stained with iron haematoxylin.

Fig. 8. Nucleus after division. Fixed with Hermann's and stained with iron haematoxylin.

Fig. 9. Ascogonial cell.

Fig. 10. Copulation of antheridium and ascogonium of same hypha by means of a copulating tube.

Fig. 11. Copulation form with the antheridium larger than the ascogonium.

Fig. 12. Projection of copulating tube.

Fig. 13. Ascogenous cells with beginning fusion. Fixed with Benda's and stained with iron haematoxylin.

Fig. 14. Copulation of lateral cells. Fixed with Hermann's and stained with iron haematoxylin.

Fig. 15. Beginning dissolution of intervening walls in tube.

Fig. 16. Nuclear fusion.

Fig. 17. Transfer of nucleus to ascogonial cell and dissolution of tube.

Fig. 18. Fertilized ascogonium with large fusion nucleus, fixed in Hermann's and stained with iodine green.

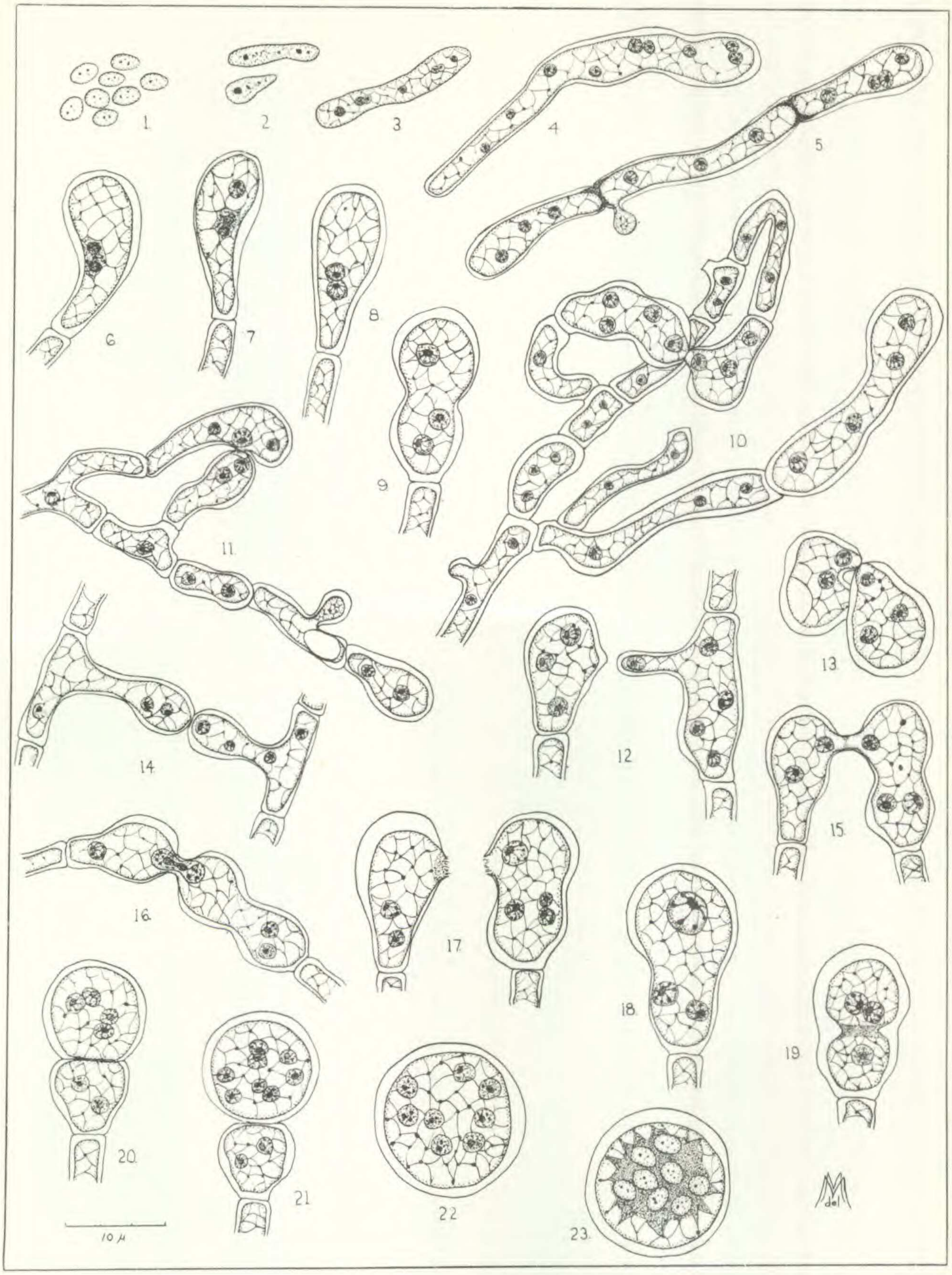
Fig. 19. Division of nucleus with beginning of formation of ascus, showing the abjunction of the special portion of the cell after syngamy. Fixed with Hermann's and stained with methylene blue.

Fig. 20. Synchronous division of nuclei. Fixed with Hermann's and stained with iron haematoxylin.

Fig. 21. Second synchronous division of nuclei with a rounding up of the future ascus.

Fig. 22. Maturation of ascus and ascospores.

Fig. 23. Matured spores in epiplasmic material.



MOORE—ENDOMYCES CAPSULATUS

Explanation of Plate

PLATE 23

All drawings made with the aid of a camera lucida and high oil-immersion magnification. × 2300.

Figs. 1–18. Grown on glycerine agar.

Figs. 19–38. Living material.

Figs. 1–9. Represent development of an ascus parthenogenetically. Fixed with Hermann's and stained with iron haematoxylin.

Fig. 10. Conidium.

Fig. 11. Cross-section of a cell showing the reticulated network.

Fig. 12. Two divided nuclei in a terminal cell.

Fig. 13. A parthenogenetically formed ascus (terminal) with spores in the center and surrounded with epiplasm.

Fig. 14. Terminal cell showing vacuoles formed by reticulum, fixed with Hermann's and stained with iodine green.

Fig. 15. Vacuolar formation in cell fixed with Hermann's and stained with methylene blue.

Fig. 16. Metachromatic granules stained with methylene blue.

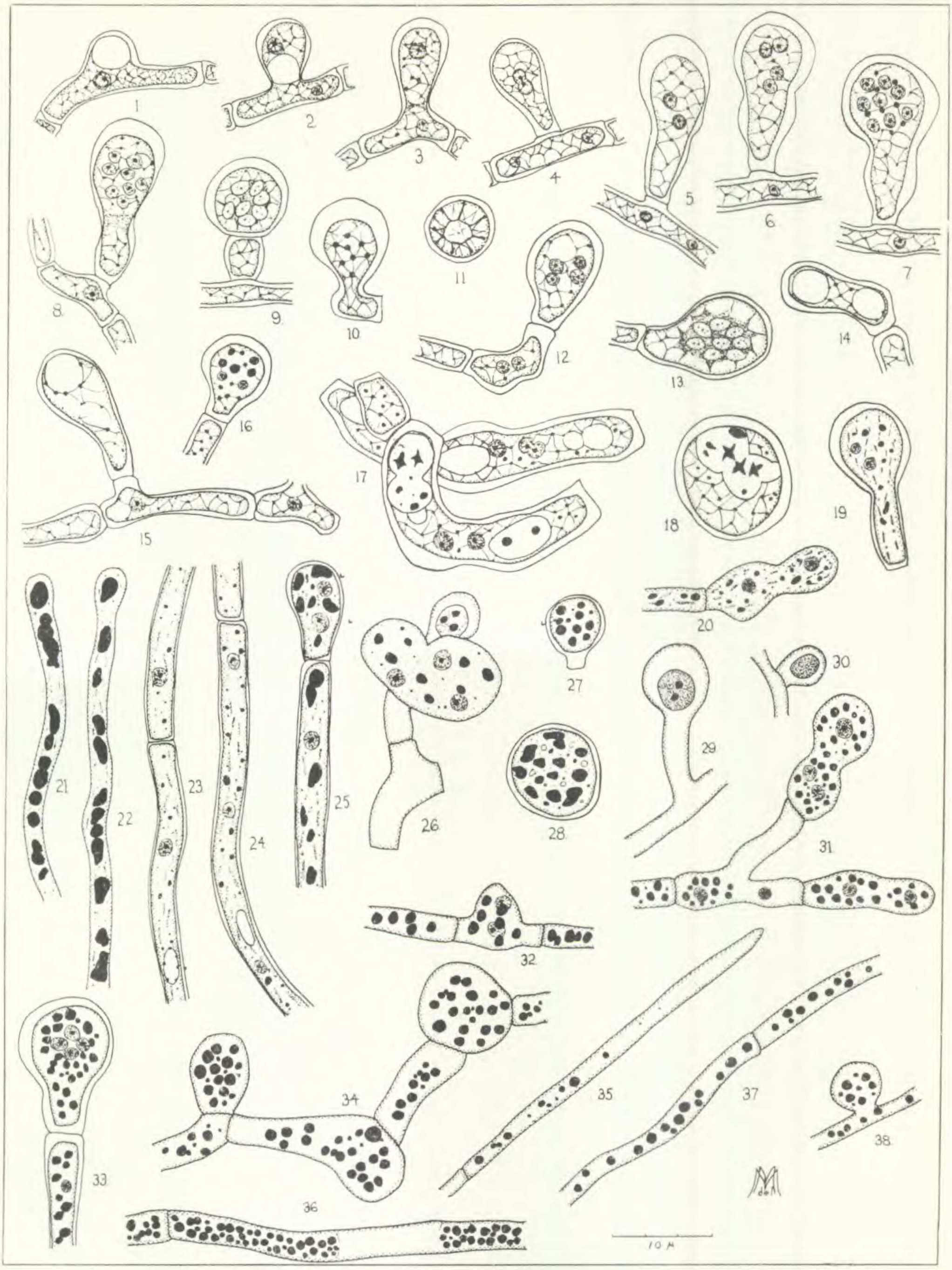
Figs. 17–18. Volutin in vacuoles.

Figs. 19–25, 27–28. Living cells stained and fixed with iodine potassium iodide, showing glycogen, lipoidal material and probable chondriosomes. The glycogen stains darkly (figs. 21–22, 25), the lipoidal substances are small, granular, and hyaline, and the chondriosomes are rod-like. Figs. 27–28 show young asci.

Figs. 26, 29–30. Living cells stained with neutral red, showing glycogen content.

Figs. 31–36. Living hyphae fixed with osmic acid, showing lipoidal or fat substances. Fig. 35, a young hypha, and fig. 36, an older hypha.

Figs. 37–38. Living material fixed with platinic chloride.



MOORE—ENDOMYCES CAPSULATUS